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Junior Member

Laurence Robert Frank Forsyth.

DEATHS

We record with regret the deaths of

Alasdair William Armstrong
Robert Karran Matthews
Owen Eldred Mott.

SCOTTISH SECTION

An Ordinary Meeting of the Section was held at 7 p.m. on Wednesday, November 28th, 1956, in the George Hotel, George Street, Edinburgh. The Chair was taken by the Chairman of the Section, Dr. F. J. Elliott, F.R.I.C., F.R.S.E.

A lecture on "Colour Chromatography (with Special Reference to Practical Development of Quantitative Methods)" was given by J. P. Cunningham, B.Sc., Ph.D., A.R.I.C.

MIDLANDS SECTION

An Ordinary Meeting of the Section was held on Tuesday, November 13th, 1956, at 7 p.m., in the Mason Theatre, The University, Edmund Street, Birmingham, 3. The Chair was taken by the Vice-Chairman of the Section, Dr. R. Belcher.

A discussion on "Laboratory Planning and Organisation" was opened by E. W. Dobson and D. Barkaway.

An Ordinary Meeting of the Section was held at 7 p.m. on Thursday, December 6th, 1956, in the Mason Theatre, The University, Edmund Street, Birmingham, 3. The Chair was taken by the Chairman of the Section, Mr. J. R. Leech, J.P.

A discussion on "Qualitative Inorganic Analysis" was opened by H. Holness, M.Sc., F.R.I.C., and R. Harrison, M.A., B.Sc., F.R.I.C.

MICROCHEMISTRY GROUP

The seventh London Discussion Meeting of the Group was held on Wednesday, November 21st, 1956, at 6.30 p.m., in the restaurant room of "The Feathers," Tudor Street, London, E.C.4. The Chair was taken by the Chairman of the Group, Dr. G. F. Hodsman, A.Inst.P.

The subject, "The Micro-determination of Sulphur," was introduced by S. Bance, B.Sc., A.R.I.C., and G. S. Crouch, and an informal discussion followed.

BIOLOGICAL METHODS GROUP

An Ordinary Meeting of the Group was held at 6.30 p.m. on Wednesday, November 14th, 1956, in the restaurant room of "The Feathers," Tudor Street, London, E.C.4. The Chair was taken by the Chairman of the Group, Mr. K. L. Smith, M.P.S.

A discussion on "Biological Methods in the General Analytical Laboratory" was opened by the Chairman.

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The Determination of Vitamin D and Related Compounds

Part I.* Introduction and Preparation of Compounds in the Irradiation Series

By W. H. C. SHAW, J. P. JEFFERIES and T. E. HOLT

(*Presented at the meeting of the Society on Wednesday, October 3rd, 1956*)

Physical and chemical methods for the determination of vitamins D are re-examined, particularly those suitable for determining the composition of the complex mixtures formed during the ultra-violet irradiation of the provitamins D (ergosterol and 7-dehydrocholesterol).

The compounds formed on irradiation may be separated into groups by preliminary chromatography on activated alumina and individual compounds then determined by ultra-violet spectroscopy and application of the antimony trichloride colour reaction. Descriptions are given of the preparation, mainly by chromatographic methods, of purified precalciferol$_2$, precalciferol$_3$, lumisterol$_2$, tachysterol$_2$ and other compounds closely related to the vitamins D. The required ultra-violet absorption characteristics and the behaviour in the colour reaction are established for each compound with these materials.

Recently the problem of determining the amounts of the vitamins D in natural or synthetic products has received considerable attention. Biological methods have been established for many years, but their well known shortcomings have led to continued search for a reliable physical or chemical method. The problem is unusually complex, since the concentration of vitamin D may vary from that of the pure vitamin (4×10^7 i.u. per g) to a few units per gram in biological material. Further, vitamin A normally accompanies vitamin D in preparations of natural origin, and it interferes to a considerable extent with most of the known methods for vitamin D. The large number of papers published in recent years on various aspects of the problem is an indication of the difficulties involved. Nevertheless, some progress has been made, particularly in the analysis of irradiation products† and in preparations derived from the pure vitamins D. In the work described in this paper we have been mainly concerned with determining the major constituents of irradiation products, although the method developed is also applicable to synthetic products in the high-potency range. The special problems of low-potency materials or interference by vitamin A are not considered.

Chemical methods for vitamins D—

The several known colour reactions given by vitamin D and the literature on them were summarised by Ewing, Kingsley, Brown and Emmett.[1] Sobel, Mayer and Kramer[2] subsequently described a method based on reaction with glycerol dichlorohydrin, Büchi and Schneider[3] re-investigated the Schaltegger method,[4] and Green devised a colorimetric method with iodine trichloride.[5] Undoubtedly the most satisfactory colorimetric method at present available involves use of the antimony trichloride reagent introduced by Brockmann and Chen[6] and subsequently improved by Nield, Russell and Zimmerli.[7] This reaction is generally considered the one most specific for vitamin D; even so, other sterols present in irradiation products give quantitatively similar intensities of colour and many compounds give much weaker colours or different ones.[8,9] Hence the reagent is of little value for the direct analysis of irradiation products, but it has been successfully applied to preparations of pure vitamin D when interfering substances were absent or could be easily eliminated.[10,11,12] It provides a sensitive and accurate method for determining certain individual irradiation products, provided these can first be separated from each other.

Physical methods for vitamins D—

An infra-red absorption method for the determination of calciferol in ergosterol irradiation products was proposed by Pirlot,[13] who measured the extinction of the relatively weak

* Presented, together with Part II, at the XVth International Congress on Pure and Applied Chemistry (Analytical Chemistry), Lisbon, September 8th to 16th, 1956.

† The phrase "irradiation products" used in this paper means the whole or part of the material obtained by submitting the provitamins D (ergosterol or 7-dehydrocholesterol) to ultra-violet irradiation.

calciferol band at about 1170 cm^{-1} by a base-line technique. This method was found relatively insensitive. The disappearance of the hydroxyl band at 1050 cm^{-1} is associated with the progressive oxidation of calciferol,[12] but absorption at this wave number is not specific for calciferol.

Apart from the suprasterol mixture produced by extended irradiation of the provitamins, and vitamin D, all the known compounds in the irradiation series, including the provitamins themselves, show selective absorption in the ultra-violet region between 250 and 310 mμ. Like the colorimetric methods, direct spectroscopic examinations of irradiation products are of little value and attempts have therefore been made to obtain chromatograph fractions of sufficient purity for application of these methods. DeWitt and Sullivan[14] used a mixture of magnesia and diatomaceous earth before a colorimetric determination. Ewing, Powell, Brown and Emmett[15] proposed Superfiltrol and determined vitamin D colorimetrically and from the extinction at 265 mμ, and Green[16] has suggested Floridin earth for the chromatographic separation. As so often happens with natural adsorbents, we found materials of the correct grade unavailable or the required degree of activity unreproducible.

Since Brockmann's[17] use of activated alumina for isolating vitamin D_3 from natural products, it appears to have received little attention, except as a means of removing oxidation products of vitamin D or unrelated interfering substances.[12] Our attention was redirected to this adsorbent by the recent work of Velluz, Amiard and Petit.[18] By chromatography of the mixed 3:5-dinitrobenzoates obtained by esterifying irradiation products prepared and freed from unchanged provitamin without the application of heat, these workers isolated the ester of a new precursor of calciferol, which they termed precalciferol$_2$; only small amounts of calciferol ester were obtained. They also described the thermal change of precalciferol$_2$ into calciferol without further irradiation and reported the development of an equilibrium between the two compounds in solutions. Precalciferol$_3$, isolated in the same way from irradiation products of 7-dehydrocholesterol, showed similar properties. These results encouraged us to re-examine the possibilities of alumina for direct chromatography of irradiation products: preliminary experiments showed little separation (except of oxidation products) on short columns, but longer ones of a suitable grade yielded fractions suitable for either colorimetric or spectroscopic analysis.

Compounds in the irradiation series—

During the preliminary work, it became evident that the behaviour in the antimony trichloride reaction of certain compounds, and their ultra-violet absorbancies, particularly those of precalciferol and tachysterol, had not been established with sufficient certainty for our purposes. It was therefore found necessary to prepare purified specimens of most of the known irradiation compounds. Published methods of preparation are laborious, but the task was made considerably easier by using on a larger scale the analytical chromatographic methods whenever they were suitable. When they differ from, or provide alternatives to, conventional methods, fuller descriptions are given below.

Experimental

Preparation of irradiated materials—

All irradiations were carried out in suitable solvents contained in 1·6-litre quartz flasks attached to a reflux condenser and exposed to the unfiltered radiations from mercury-vapour lamps. Arrangements were made to keep the contents of certain flasks cold during irradiation, by circulating cold water, or other suitable cooling fluid, over the outsides.

After irradiation, the solvent was distilled, unchanged provitamin (ergosterol or 7-dehydrocholesterol) being then removed by treatment with diluted ethanol. The residual provitamin in the resulting resin was normally reduced to less than 4 per cent. by this treatment; smaller amounts caused no interference in the subsequent chromatography. Many irradiation compounds appear to be more or less thermolabile or sensitive to oxidation, particularly when impure. When it was not possible to use crude materials immediately after preparation, they were stored in small, stoppered, nitrogen-filled flasks and kept on solid carbon dioxide.

Chromatography—

For most preparative purposes, chromatograph tubes 60 cm long and having an external diameter of 4·2 cm proved satisfactory. Each tube was provided with a B40 socket at the

top and the tapered lower end was fused to a short length of narrow-bore tubing. The tubes were two-thirds filled with light petroleum, boiling range 40° to 60° C, and activated alumina of the correct grade was poured through the solvent to form a 50-cm column, on which up to 10 g of material could be chromatographed.

Details of preparing the columns, of grading the alumina and of solvents and necessary apparatus and materials are given in Part II (p. 8). The chromatographic behaviour of the various irradiation compounds on the small columns used for analytical purposes is also described in Part II.

COMPOUNDS IN THE VITAMIN-D_2 SERIES—

Ergosterol—Purified by recrystallisation of the benzoate and regeneration of the sterol, as described earlier.[19]

Lumisterol$_2$—Isolated as the 3:5-dinitrobenzoate from the mother liquors remaining after the commercial crystallisation of calciferol ester. After saponification the free sterol was recrystallised several times and then dried *in vacuo* at 100° C; this solid had m.p. 118° to 119° C and $[\alpha]_D^{20} + 186°$ (2 per cent. w/v in acetone).

Tachysterol$_2$—Prepared by irradiation of ergosterol in ether and subsequent chromatographic separation. Obtained crystalline as the 4-methyl 3:5-dinitrobenzoate and as the 3:5-dinitrobenzoate. 4-Methyl 3:5-dinitrobenzoate: m.p. 155° to 156°C; $E_{1cm}^{1\%}$ at 280 mμ ($\lambda_{max.}$) in ethanol = 505. 3:5-Dinitrobenzoate: on analysis, C, 71·6%; H, 8·00%; N, 4·82% ($C_{35}H_{46}O_6N_2$ requires C, 71·2%; H, 7·85%; N, 4·74%); m.p. 129° to 131° C; $E_{1cm}^{1\%}$ at 280 mμ ($\lambda_{max.}$) in ethanol = 523.

Precalciferol$_2$—Small quantities of the free steroid were prepared by refluxing calciferol in benzene for 30 minutes. After chromatographic separation from unchanged calciferol, the precalciferol$_2$ fraction was either used as such or distilled to dryness below 20° C, residual solvent being removed in a high vacuum. As precalciferol$_2$ is thermolabile and sensitive to oxidation, samples so prepared were used immediately.

Precalciferol$_2$ was also obtained as the stable crystalline 3:5-dinitrobenzoate from irradiations of ergosterol carried out below 20° C. The crude irradiation product after removal of unchanged ergosterol was chromatographed on alumina; the precalciferol$_2$ - lumisterol$_2$ fraction was esterified with 3:5-dinitrobenzoyl chloride in the cold, and the precalciferol$_2$ ester separated by crystallisation. This was recrystallised from a mixture of ethyl methyl ketone and ethanol.[18] Precalciferol$_2$ 3:5-dinitrobenzoate had m.p. 100° to 101° C and $[\alpha]_D^{20} + 44°$ (1 per cent. w/v in chloroform).

Calciferol (*ergocalciferol*)—Material of pharmaceutical quality was used in the irradiation studies, in the recovery experiments described in Part II and for the preparation of the pyrocalciferols.

Pyrocalciferol$_2$—Both pyro- and *iso*pyro-calciferol$_2$ were obtained by heating 6 g of calciferol in a sealed nitrogen-filled ampoule at 180° C for 18 hours. Chromatography on the large column gave good separation into three fractions. The first, 3·85 g (64 per cent.), consisted mainly of pyrocalciferol$_2$, together with some by-products; spectroscopic results calculated from subsequently established figures for the purified compound indicated 60 per cent., calculated on the starting material. This fraction could not be crystallised and was therefore converted to the 3:5-dinitrobenzoate. The resulting ester, obtained crystalline, was saponified and the regenerated sterol recrystallised from aqueous acetone; this solid had m.p. 93° to 94° C and $[\alpha]_D^{20} + 495°$ (0·25 per cent. w/v in absolute ethanol); on analysis, C, 86·4%; H, 11·21% ($C_{28}H_{44}O$ requires C, 84·8%; H, 11·18%).

iso*Pyrocalciferol*$_2$—The second fraction, 0·20 g (3·2 per cent.), obtained in the chromatography of the crude pyrocalciferols consisted mainly of unchanged calciferol and the third fraction, 1·97 g (33 per cent.), of *iso*pyrocalciferol$_2$ in a sufficient state of purity for direct crystallisation from 95 per cent. v/v ethanol. Spectroscopic results on this fraction gave 31 per cent., calculated on the original weight of calciferol; it had m.p. 112° to 116° C and $[\alpha]_D^{20} + 320°$ (0·5 per cent. w/v in chloroform); on analysis, C, 83·2%; H, 11·16% ($C_{28}H_{44}O$ requires C, 84·8%; H, 11·18%).

Suprasterols$_2$—Suprasterols$_2$ I and II, prepared by extended irradiation of calciferol until no selective ultra-violet absorption above 250 mμ remained, were isolated as the mixed allophanates.[20] Suprasterol$_2$-I allophanate could not be obtained crystalline and the material was therefore saponified and chromatographed on a 100-cm × 2·3-cm column. This length of column was found necessary for separating suprasterol$_2$ I from suprasterol$_2$ II, apparently

the main impurity. The suprasterol$_2$-I fraction was distilled to dryness and the residue, although not quite pure steroid, used without further treatment.

Suprasterol$_2$-II allophanate was obtained crystalline from the mother liquors remaining after the precipitation[20] of suprasterol$_2$ I and then recrystallised from a mixture of ethyl acetate and methanol; it had m.p. 224° to 226° C. and $[\alpha]_D^{20} + 76°$; on analysis, C, 73·1%; H, 9·41% ($C_{29}H_{44}O_3N_2$ requires C, 74·4%; H, 9·47%). The free steroid was regenerated as required by saponification of portions of this material.

COMPOUNDS IN THE VITAMIN-D_3 SERIES—

All the compounds in the full vitamin-D_3 series have not been prepared. There is, however, no indication that they cannot be made by the same methods, nor that any error is introduced by using for analytical purposes the same ultra-violet molecular extinction values as for the corresponding compounds in the ergosterol series.

Vitamin D_3 (cholecalciferol)—Crystalline material obtained by saponification of the purified 3:5-dinitrobenzoate.

Precalciferol$_3$—Small quantities were prepared as required from vitamin D_3 in the manner described for precalciferol$_2$. The 3:5-dinitrobenzoate was prepared by esterification of this material with 3:5-dinitrobenzoyl chloride in the cold and the ester was then purified by recrystallisation. Precalciferol$_3$ 3:5-dinitrobenzoate had m.p. 104·5° to 105° C and $[\alpha]_D^{20} + 54°$ (1 per cent. w/v in chloroform).

The absorption spectra for the compounds in the calciferol (vitamin-D_2) series are given in Figs. 1(*a*) to 1(*f*) and the extinction values for all compounds prepared in Table I. The shapes of the absorption curves for the unstable compounds, tachysterol$_2$ and precalciferol$_2$, have been taken from the purest specimens prepared; the extinction values given for these two compounds in Table I have been obtained by subtracting from those of the 4-methyl 3:5-dinitrobenzoate or the 3:5-dinitrobenzoate, respectively, the appropriate values for the corresponding *cyclo*hexyl esters. Table I also gives the apparent intensities of colour developed by each of the compounds when assayed by means of the antimony trichloride reagent in the manner described in Part II. Vitamins D_2 and D_3 have each been taken as 100 in the respective parts of the table.

TABLE I

ULTRA-VIOLET ABSORPTION OF VITAMINS D AND RELATED COMPOUNDS IN ETHANOL

Compound	Wavelength (± 0·5 mμ), mμ	$E_{1cm}^{1\%}$	Molecular extinction coefficient	Antimony trichloride colour
Ergosterol (anhydrous)	271·5 (max.)	290	11,500	less than 1%
	282·0 (max.)	306	12,150	(yellow)
	293·5 (max.)	174	6900	
Lumisterol$_2$	260·0*	170	6750	less than 1%
	272·0 (max.)	238	9450	(yellow)
	280·0* (inflex.)	224	8900	
Precalciferol$_2$	260·0 (max.)	230	9150	100% (orange)
	280·0*	153	6050	
Tachysterol$_2$	265·0*	490	19,450	96 to 100%
	271·0 (inflex.)	609	24,150	(orange)
	280·5 (max.)	745	29,550	
	289·5 (inflex.)	608	24,100	
Calciferol (Vitamin D_2, ergocalciferol) ..	265·0 (max.)	475	18,850	100% (orange)
	280·0*	341	13,550	
Pyrocalciferol$_2$	274·0 (max.)	259	10,250	less than 1%
	284·5 (max.)	273	10,850	(yellow)
	296·0 (max.)	161	6400	
*iso*Pyrocalciferol$_2$	274·0 (max.)	261	10,350	less than 1%
	284·0 (max.)	259	10,250	(yellow)
	295·0 (inflex.)	142	5650	
Suprasterols$_2$ I and II	Negligible absorption above 250 mμ			less than 1%
	No selective absorption above 220 mμ			(yellow)
Vitamin D_3 (cholecalciferol)	265·0 (max.)	490	18,850	100% (orange)
	280·0*	351	13,550	
Precalciferol$_3$	260·0 (max.)	240	9250	100% (orange)
	280·0*	159	6100	

* Extinction values at these wavelengths are required for the calculations described in Part II. The presence of a maximum or minimum is not implied.

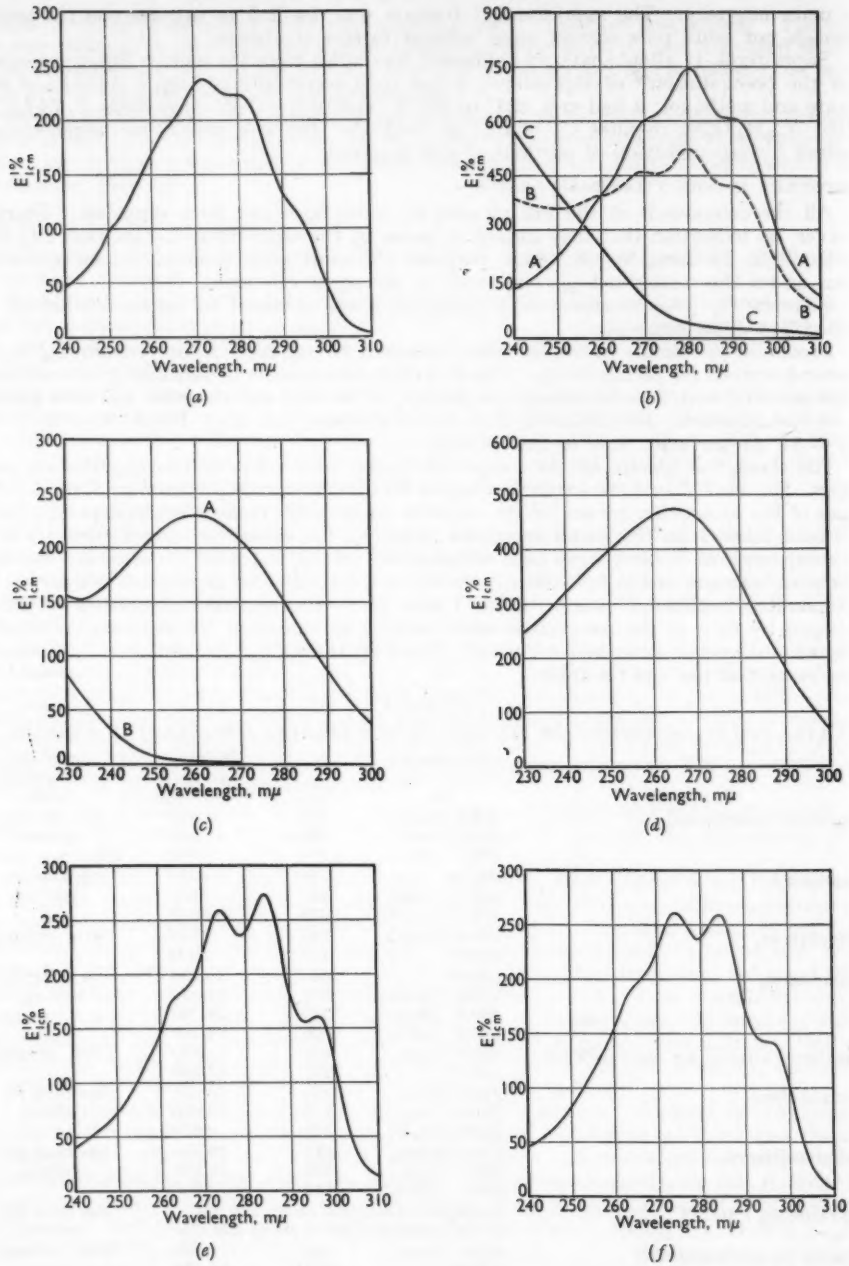

Fig. 1. Absorption spectra (in absolute ethanol at concentrations of 0·001 to 0·002 per cent.). (*a*) Lumisterol$_2$. (*b*) Curve A, tachysterol$_2$; curve B, tachysterol$_2$ 3:5-dinitrobenzoate; curve C, *cyclo*hexyl 3:5-dinitrobenzoate. (*c*) Curve A, precalciferol$_2$; curve B, suprasterol$_2$ I. (*d*) Calciferol. (*e*) Pyrocalciferol$_2$. (*f*) *iso*Pyrocalciferol$_2$

The extinction value found for precalciferol$_2$ agrees well with the result obtained by Velluz *et al.*,[18] but we find maximum extinction at 260 mμ, as against 265 mμ, the position found by subtraction from the curve for precalciferol 3:5-dinitrobenzoate; this appears to be an unreliable procedure, because of the high and steeply rising contribution from the 3:5-dinitrobenzoate part of the molecule. Our value of 260 mμ is in better agreement with the 262 mμ quoted subsequently by Inhoffen, Bruckner, Irmscher and Quinkert.[21] This point is of particular interest in relation to the validity of the different structures recently proposed for precalciferol.[22,23,24,25,26,27]

The value for vitamin D$_3$ at 265 mμ (max.) is that quoted for the International Standard.[28]

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Glaxo Laboratories Ltd.
Greenford, Middx.

June 7th, **1956**

The Determination of Vitamin D and Related Compounds

Part II.* Analysis of Irradiation Products

By W. H. C. SHAW and J. P. JEFFERIES

(*Presented at the meeting of the Society on Wednesday, October 3rd, 1956*)

The mixture of compounds formed during the irradiation of the provitamins D may be separated into two main groups by chromatography on a suitable grade of activated alumina and development of the chromatogram with a mixture of light petroleum and acetone; individual compounds may then be determined in each group by ultra-violet absorption and colorimetric methods. A method is also given for the separate determination of the residual provitamins D.

Products obtained by irradiating the provitamins under different conditions are examined by the methods described and the results are discussed in relation to the structural changes that take place during, and subsequent to, irradiation and in relation to the results of biological assays.

The methods are also applicable to the determination of the precalciferols and the vitamins D in preparations of the pure vitamins D, but in their present form are unsuitable for materials of low potency or those containing vitamin A.

In Part I[1] of this series we have briefly discussed the chemical and physical methods available for the determination of vitamin D. Of these, we have selected the well known colour reaction with antimony trichloride and ultra-violet absorption as being suitable for our purposes. Little useful information can be obtained by the direct application of either method to the complex and unstable mixtures produced by irradiation of the provitamins D. If, however, such mixtures are broken down quantitatively into simpler fractions, each containing only one or two of the "active" components, the two methods can be used in conjunction for the determination of individual compounds. This has been achieved by chromatography on grade III (Brockmann) activated alumina with 6 per cent. v/v of acetone in light petroleum, boiling range 40° to 60° C, as eluent.

The method described in this paper is applicable to high-potency preparations of synthetic vitamins D as well as to irradiation products, but is unsuitable in its present form for materials containing vitamin A or for samples of low potency.

The preparation of purified individual compounds of the ergosterol irradiation series was described in Part I and with these materials the necessary colorimetric and spectroscopic standards were established.

Methods

Apparatus—

Chromatograph tubes—Made from Pyrex-glass tubing, 55 cm long and of external diameter 1·4 cm. Each tube is fitted with a tap and the upper end is sealed to a length of tubing (10 cm × 2 cm) carrying a B19 ground-glass socket.

Solvent reservoirs—Standard-joint (B24 or B19) 500-ml or 1-litre separators, each provided with a B19 cone on the stem.

Constant-pressure device—Air pressure is applied to the tops of the solvent reservoirs by means of a small electric pump. The pump is connected to a T-piece, one arm of which is provided with an air-leak controlled by a screw clip and the other connected through a capillary to a large reservoir that serves to even out pulsations. A water manometer about 1 metre in height is connected to the circuit and in use a pressure of about 50 cm of water is applied, although this may be varied within wide limits without affecting the efficiency of separation. All joints and taps on the apparatus must be fitted with rubber bands or other means taken to ensure that they withstand the pressure applied.

* Presented, together with Part I, at the XVth International Congress on Pure and Applied Chemistry (Analytical Chemistry), Lisbon, September 8th to 16th, 1956.

Column filling tube—One end of a 35-cm tube having a diameter of 2 cm is provided with a B19 cone and the other is fused to a wide funnel made from the top of a 150-ml conical flask. A side-arm fitted as near as possible to the ground-glass joint is closed with a short rubber tube and a spring clip.

*Automatic fraction collector**—Adjusted to collect 6-g (approximately 9-ml) fractions.

Ultra-violet spectrophotometer—A Unicam SP500 or other suitable instrument. The wavelength and optical-density scales should be checked in the manner previously described.[2]

Photo-electric absorptiometer—A Spekker absorptiometer provided with glass-covered 1-cm cells, Spectrum blue-green (No. 603) and heat-absorbing filters.

When the instrument is used with antimony trichloride reagent, the lens of the instrument and filters should be cleaned frequently.

Glass cups—Small cups, about 1 cm in height and of the same diameter, cut from square-base specimen tubes.

MATERIALS—

Activated alumina—Expose activated alumina† in thin layers to a moist atmosphere until the required degree of activity is reached. Alternatively, water may be added directly to the alumina, provided mixing is thorough and equilibrium is attained. Test for activity in 5-cm × 1·2-cm columns by the method of Brockmann and Schodder[3] with 10 ml of test solution No. 3 (Sudan red and Sudan yellow, 0·04 per cent. w/v of each dye in a solvent prepared by mixing one volume of benzene and four volumes of light petroleum, boiling range 40° to 60° C). After development with 20 ml of the solvent, the front of the lower (Sudan yellow) band should be 3 to 4 cm from the top of the column.

Solvents—Commercial grades of acetone and of light petroleum, boiling range 40° to 60° C, redistilled in all-glass apparatus before use, were found satisfactory.

DETERMINATION OF RESIDUAL PROVITAMIN—

Small amounts of ergosterol and 7-dehydrocholesterol are normally separated from the other active compounds during the chromatography described below, but their determination is generally more satisfactorily carried out directly. They are the only compounds related to the vitamins D that are precipitated quantitatively from diluted ethanol by digitonin.

*iso*Pyrocalciferol has a configuration similar to that of the provitamins and is precipitated to some extent. Neither pyro- nor *iso*pyro-calciferol normally occurs in irradiation products, even in those prepared in relatively high-boiling solvents, so that interference from this source is unlikely.

PROCEDURE—

Weigh a portion of the sample containing about 1 mg of provitamin into a stoppered 10-ml test-tube. Dissolve in 5 ml of absolute ethanol and add an equal volume of 0·5 per cent. w/v digitonin in 80 per cent. v/v ethanol. Warm to about 70° C and set aside overnight. Collect the precipitate by centrifugation and wash it several times with precipitant. Dissolve the residue in absolute ethanol and determine its extinction values at the 271·5, 282 and 293·5-mμ maxima and at 310 mμ. The ratio of the extinctions should agree with those for pure ergosterol,[4] but, if not, the digitonide may be purified by dissolving it in absolute ethanol and re-precipitating it with digitonin solution.

$E^{1\%}_{1cm}$ at 282 mμ ($\lambda_{max.}$) for anhydrous ergosterol in absolute ethanol is 306 and for 7-dehydrocholesterol, 315.

The results given in Table I for known mixtures containing ergosterol show satisfactory recovery.

CHROMATOGRAPHY

On the particular grade of alumina selected a relatively wide choice of solvents is available for development. The particular combination chosen gives a satisfactory rate of development: it is not too volatile for a pipette to be used, yet small quantities can easily be evaporated to dryness without heating.

* Obtainable from the Shandon Scientific Company, London.

† Grade O (obtainable from Peter Spence & Sons Ltd., Widnes).

TABLE I

RECOVERY OF ERGOSTEROL IN THE PRESENCE OF VITAMIN-D_2 RESIN

Resin taken, mg	Ergosterol added, mg	Total ergosterol found, mg	Recovery of added ergosterol, %
19·6	—	0·116	—
95·4	—	0·668	—
20·1	0·36	0·474	96
20·0	0·72	0·826	97

PREPARATION OF COLUMNS—

Place a small plug of cotton-wool in the bottom of a chromatograph tube, attach the filling tube, close the side-arm of the tube, and fill with light petroleum, boiling range 40° to 60° C, to the bottom of the conical portion. Pour the prepared activated alumina slowly in a thin stream through the filling tube until a 50-cm column is obtained. Empty the filling tube through the side-arm, remove it and place a small disc of filter-paper on top of the alumina.

The apparatus should be placed away from strong light.

PREPARATION OF SAMPLES—

Freshly prepared vitamin-D resins‡ are generally completely soluble in light petroleum, boiling range 40° to 60° C, and a solution of the sample in this solvent should be prepared such that up to 300 mg (containing not more than 150 mg of "active" compounds) are contained in 50 ml. No heat may be used in preparing the solution. Resins that have been over-irradiated or have become oxidised may not be completely soluble. These should be dissolved in the minimum amount of acetone and the solution then diluted to 50 ml with light petroleum, boiling range 40° to 60° C. A turbidity may occur on dilution, but the insoluble matter is retained firmly on the top of the column and does not affect the efficiency of separation.

If only small amounts of material are available, up to 30 mg may be dissolved directly in 5 ml of light petroleum, boiling range 40° to 60° C, but whenever possible the aliquot procedure should be followed.

DEVELOPMENT OF CHROMATOGRAM—

Place 5·00 ml of the sample solution on the prepared column and wash in with several 1-ml portions of light petroleum, boiling range 40° to 60° C. Attach the reservoir filled with light petroleum containing 6 per cent. v/v of acetone to the chromatograph tube and elute at about 2 ml per minute by applying air pressure to the top of the reservoir.

Reject 45 ml of eluate and then collect 6-g fractions until the end of the calciferol band is reached (see below). Test the fractions by evaporating 0·05 ml of each in separate glass cups to dryness in a stream of air. From a burette add about 0·5 ml of antimony trichloride reagent to each cup.

TABLE II

APPROXIMATE CHROMATOGRAPHIC BEHAVIOUR OF CALCIFEROL AND RELATED COMPOUNDS

		Approximate fractions Nos.*
Precalciferol$_2$	Precalciferol band	5 to 11
Lumisterol$_2$		
Suprasterol$_2$ II (partly)		
(Pyrocalciferol$_2$)†		
Calciferol	Calciferol band	14 to 23
Tachysterol$_2$		
Suprasterol$_2$ I (partly)		
Ergosterol	—	24 to 31
(*iso*Pyrocalciferol$_2$)†		

* 50-cm × 1·3-cm column, 45 ml of eluate rejected, 6-g (≡ 9-ml approximately) fractions.

† These compounds are not normally present.

‡ This term is used for brevity to describe the non-crystalline solvent-free mixture of solids resulting from the irradiation of either provitamin D after removal of most of the unchanged provitamin.

Table II gives the approximate positions in which the various irradiation products are eluted. If desired, small alterations in the percentage of acetone in the developing solvent can be made to compensate for small variations in the activity of the alumina.

Separation should be clear-cut, with one to three negative fractions between the two bands. Overlapping only normally occurs if the column has been overloaded. Transfer the fractions comprising the first (precalciferol) band quantitatively to a 100-ml calibrated flask and dilute to volume with light petroleum. Dilute the second (calciferol) band similarly to 150 ml or other convenient volume for further examination, which should be carried out immediately.

ANALYSIS OF THE PRECALCIFEROL BAND—

This band contains precalciferol and lumisterol. It may also contain suprasterols, which do not interfere with the subsequent determinations.

The aliquots given below are intended only as a guide and should be varied according to the amounts of the two compounds in the sample.

(a) *Ultra-violet absorption*—By pipette put a suitable aliquot (5 ml) into a 25-ml conical flask and evaporate the solvent in a stream of oxygen-free nitrogen. Dissolve the dry residue in an accurately measured volume (10 ml) of absolute ethanol. Mix, and measure the extinctions of the solution in a 1-cm cell at 260 mμ and 280 mμ, against the plain solvent. If necessary, a full curve may be plotted as a qualitative check.

Calculate the observed extinctions in terms of $E^{1\%}_{1cm}$ values on the weight of sample taken for chromatography, and then calculate the percentages of precalciferol and lumisterol from the following formulae—

precalciferol$_2$, per cent. = 0·8780 $E^{1\%}_{1cm}$ at 260 mμ − 0·6661 $E^{1\%}_{1cm}$ at 280 mμ;
lumisterol$_2$, per cent. = 0·9014 $E^{1\%}_{1cm}$ at 280 mμ − 0·5994 $E^{1\%}_{1cm}$ at 260 mμ;
precalciferol$_3$, per cent. = 0·8514 $E^{1\%}_{1cm}$ at 260 mμ − 0·6460 $E^{1\%}_{1cm}$ at 280 mμ;
lumisterol$_3$, per cent. = 0·8740 $E^{1\%}_{1cm}$ at 280 mμ − 0·5811 $E^{1\%}_{1cm}$ at 260 mμ.

(b) *Colorimetric examination*—By pipette put suitable aliquots (1 ml) into each of two 25-ml conical flasks and evaporate to dryness in a stream of oxygen-free nitrogen. Dissolve the residue in 1·00 ml of analytical-reagent grade chloroform, and complete the duplicate colorimetric determinations with antimony trichloride reagent, as described below. Calculate the percentage of precalciferol in the sample.

ANALYSIS OF THE VITAMIN-D BAND—

This band contains calciferol and tachysterol. It may also contain suprasterol II, which does not interfere in the determinations. The provitamins (ergosterol or 7-dehydrocholesterol) are eluted immediately after this band, and care should be taken to exclude the subsequent chromatograph fractions that are negative or very faintly yellow (ergosterol) or purple (7-dehydrocholesterol) to antimony trichloride reagent.

(a) *Ultra-violet absorption*—Evaporate a suitable aliquot (2 ml) of the vitamin-D band to dryness in nitrogen, dissolve the residue in a pipette-measured volume (10 ml) of absolute ethanol, and determine the extinction values at 265 mμ and 280 mμ in a 1-cm cell against the plain solvent.

Calculate the $E^{1\%}_{1cm}$ values for the sample taken, and then calculate the percentages of vitamin D and tachysterol from the following formulae—

calciferol, per cent. = 0·3988 $E^{1\%}_{1cm}$ at 265 mμ − 0·2624 $E^{1\%}_{1cm}$ at 280 mμ;
tachysterol$_2$, per cent. = 0·2544 $E^{1\%}_{1cm}$ at 280 mμ − 0·1826 $E^{1\%}_{1cm}$ at 265 mμ;
vitamin D$_3$, per cent. = 0·3867 $E^{1\%}_{1cm}$ at 265 mμ − 0·2545 $E^{1\%}_{1cm}$ at 280 mμ;
tachysterol$_3$, per cent. = 0·2466 $E^{1\%}_{1cm}$ at 280 mμ − 0·1770 $E^{1\%}_{1cm}$ at 265 mμ.

(b) *Colorimetric examination*—Carry out duplicate colorimetric determinations on the residue from suitable aliquots (1 ml), and calculate the result as the apparent percentage of vitamin D in the sample. The figure so obtained is the sum of the vitamin D and tachysterol contents.

This total figure normally agrees well with the sum of the spectroscopic results for vitamin D and tachysterol: any appreciable discrepancy suggests the presence of some provitamin.

Method for antimony trichloride - acetyl chloride colorimetric assay

Reagents—

Antimony trichloride - acetyl chloride reagent—Prepare as described by Nield, Russell and Zimmerli.[5] When stored in filled 100-ml glass-stoppered flasks kept in the dark, the reagent is stable for several months.

Chloroform—Analytical-reagent grade material is satisfactory for preparing both test solutions and standard solutions for calibration purposes; B.P. quality chloroform proved unsatisfactory.

Procedure—

Dissolve duplicate portions of the residue for examination in 1·00 ml of analytical-reagent grade chloroform, and add from a burette 9·00 ml of antimony trichloride reagent. Mix by swirling, fill a 1-cm absorptiometer cell with the solution and cover with a glass slide. Measure the optical density of the solution exactly 2 minutes after mixing and again after 3 minutes, with water in the comparison cell and with Spectrum blue-green (No. 603) and heat-absorbing filters.

Calculate the mean of the four readings and deduct any blank for the reagent measured against water. From a calibration graph read off the amount of calciferol (vitamin D_3, or precalciferol, as the case may be—see Note 2) present, and calculate this as a percentage of the sample.

Prepare a calibration graph with 0·25, 0·50, 0·75 and 1·00-ml amounts of a solution of the purified standard (40 μg per ml) in analytical-reagent grade chloroform, adding sufficient reagent to each to produce 10·00 ml. The graph relating concentration to optical density should be rectilinear and pass through the origin.

The slope of the graph varies somewhat from day-to-day; to obtain the required accuracy, it is essential to check at least one point on the upper portion of the graph daily.

Notes—1. Reliable results cannot be obtained by adding the reagent directly to the residue to be tested.

2. Purified calciferol or vitamin D_3 should be used, as appropriate, for preparing the calibration graph.

Recovery of known compounds—

In view of earlier criticisms of incomplete recovery on alumina, we thought it desirable to check in detail recovery of the various compounds in the irradiation series on the grade of alumina used throughout this work.

The unstable compounds, tachysterol$_2$ and precalciferol$_2$, were prepared as solutions from the ester and calciferol, respectively, immediately before the experiments. The solutions were assayed spectroscopically and colorimetrically before and after chromatography, and the recoveries are calculated on this basis. The remaining compounds were added as aliquots of known solutions.

The results, given in Table III, show good recovery of most compounds. Part of the loss is due to removal of small amounts from the various fractions for testing purposes, and small losses also occur during operation of the automatic fraction collector.

Up to about 80 to 85 per cent. of the tachysterol$_2$ is recovered; attempts to improve this on weaker grades of alumina and with weaker eluents were unsuccessful. The proportion lost appears to be independent of the amount put on the column.

The small amount of calciferol found in experiment 4 is due to a small change of precalciferol$_2$ into calciferol during the time required for chromatography.

The apparent tachysterol$_2$ found in experiment 7 is due to incomplete separation of the relatively large proportion of ergosterol added; the small amount remaining in the calciferol band affected the spectroscopic, but not the colorimetric, results.

TABLE III

RECOVERY OF IRRADIATION COMPOUNDS

	Compound	Weight taken, mg	Recovery by spectroscopic method, %	Recovery by colorimetric method, %
1.	Ergosterol	4·9	98	—
2.	Lumisterol$_2$	10·0	98	—
3.	Precalciferol$_2$	0·96	99	96
4.	Precalciferol$_2$	19·9	96	99
	Calciferol	nil	1	1
5.	Calciferol	10·0	99	97
6.	Calciferol	1·23	98	96
7.	Ergosterol	2·1	—	—
	Calciferol	9·7	99	98
	Tachysterol$_2$	nil	4*	—
8.	Tachysterol$_2$	0·80	81	83
9.	Tachysterol$_2$	3·37	85	88
10.	Tachysterol$_2$	16·9	83	82
11.	Calciferol	2·06	95	90
	Tachysterol$_2$	1·27	78	—
12.	7-Dehydrocholesterol	4·9	96	—
13.	Precalciferol$_3$	16·8	100	99
	Vitamin D$_3$	nil	less than 1	—
14.	Vitamin D$_3$	1·10	99	96
15.	Vitamin D$_3$	10·0	98	99

* Result due to incomplete separation; for explanation see text.

RESULTS AND DISCUSSION

Some hundreds of samples have been examined by the methods described. Most of these received a variety of treatments either during irradiation or subsequently, and it would be inappropriate to give figures for such samples here. The results given in Table IV have accordingly been confined to typical examples of irradiation products prepared in ether or benzene, the two most commonly used solvents.

The results on samples Nos. 1 and 7 show that, in both the ergosterol and 7-dehydrocholesterol series, the precalciferols are the main products of irradiation when this and subsequent operations are carried out without heating; only very small amounts of the vitamins themselves are formed, these undoubtedly arising by thermal change (the so called "dark reaction") from the precalciferols during the time required for preparation and chromatography. The results amply confirm those of Velluz, Amiard and Petit,[6] who obtained precalciferols as the main products when processing was carried out in the cold, and show that the vitamins D are not directly in the irradiation series. Samples 8 and 11 show the effect of forming the equilibrium mixture by refluxing in benzene, and 9 shows the partial change during storage at lower temperatures. The results for Nos. 2, 3 and 5, given increasing times of irradiation, show that the total of precalciferol and calciferol in the resulting resin is not greatly increased by prolonging the irradiation time, though the proportion of provitamin changed (not shown in Table IV) is naturally greater for the longer irradiation periods.

As a further check on the validity of the methods described here, the ultra-violet absorption curves and the antimony trichloride colours were determined directly on samples 5 and 9, which contained a preponderance of precalciferol$_2$ and vitamin D$_3$, respectively. The various fractions obtained chromatographically from these two samples were then examined in the same way. The gross absorption curves are shown in Figs. 1 and 2, curves A; in each figure, E represents the sum of B, C and D, the absorption spectra of the various fractions. The differences between E and A are almost entirely accounted for by the loss of tachysterol during chromatography. Moreover, the shapes of the curves B and C for the precalciferol and calciferol fractions, respectively, show good agreement with the theoretical curves (indicated by small crosses) calculated from the appropriate results for the individual compounds in the two fractions. The small differences found at short wavelengths do not affect the results calculated from observations at or above 260 mμ.

TABLE IV

COMPOSITION OF VITAMIN-D RESINS

Sample No.	Lumisterol,* %	Precalciferol,* %	Vitamin D,* %	Tachysterol,*† %	Notes‡
1	8·9 10·2	52·6 52·4	1·9 — 14·6	12·7	5 g of ergosterol. Irradiated for 90 minutes at 0° C under nitrogen. Processed cold.
2	5·9 6·0	7·6 7·6	37·2 — 42·3	5·3	20 g of ergosterol. Irradiated for 30 minutes at boiling point. Equilibrium mixture made in diluted ethanol at boiling point. Residual ergosterol 2·7%.
3	9·1 8·9	45·1 42·8	6·7 — 13·8	7·9	20 g of ergosterol. Irradiated for 90 minutes at boiling point.
4	9·5 9·6	12·0 11·9	41·1 — 53·5	11·8	20 g of ergosterol. Irradiated for 90 minutes at boiling point. Refluxed in diluted ethanol to form equilibrium mixture.
5	10·5 12·1	31·0 29·9	19·0 — 31·7	12·9	20 g of ergosterol. Irradiated for 6 hours at boiling point.
6	11·7 9·9	14·5 15·8	43·7 — 45·7	3·5	20 g of ergosterol in 1·4 litres of benzene at boiling point. Precalciferol - lumisterol fraction slightly impure.
7	3·2 1·7	56·7 57·8	1·3 — 12·6	11·5	10 g of 7-dehydrocholesterol. Irradiated and processed cold. Residual 7-dehydrocholesterol 9·8%.
8	2·9 3·2	10·9 10·6	44·4 — 55·4	10·8	No. 7 refluxed in benzene to form equilibrium mixture.
9	2·5 2·5	13·4 13·3	42·6 — 55·8	12·9	No. 7 stored under nitrogen at room temperature (20° C) for 7 days.
10	5·3 5·6	33·7 34·6	16·9 — 27·7	11·4	20 g of 7-dehydrocholesterol. Irradiated for 90 minutes at boiling point.
11	6·7 6·5	8·8 9·3	37·4 — 49·8	12·2	No. 10 refluxed in benzene to form equilibrium mixture.

* The upper figures for each sample were obtained spectroscopically, the lower figures colorimetrically. The lower figures for lumisterol were calculated from the optical density at 270 mμ corrected for precalciferol (determined colorimetrically).

† Not corrected for loss during chromatography.

‡ Unless indicated otherwise all irradiations were done in 1·4 litres of ether in quartz flasks with the unfiltered radiation from mercury-vapour lamps. "Processing" here means removal of unchanged provitamin by treatment with diluted ethanol, or by other suitable treatment.

The colorimetric values for these same samples also showed substantially quantitative recovery, although a small amount of matter reactive to antimony trichloride reagent was found firmly retained on the top few millimetres of the column, even after elution with 25 per cent. v/v of ethanol in acetone.

Irradiation products prepared in higher boiling solvents (*e.g.*, No. 6, irradiated in benzene) contain vitamin D itself as the major component, since at higher temperatures the change from precalciferol to the corresponding vitamin proceeds to a considerable extent during irradiation. When this change takes place while the solution is being irradiated (that is, when the actual vitamin is one of compounds irradiated), there is evidence that traces of compounds not included in the present scheme of analysis are formed; these are eluted from the chromatograph column with the precalciferol - lumisterol band and interfere to a small extent with both colorimetric and spectroscopic results.

In Table V the analytical figures for a vitamin-D_2 resin (prepared without heating) are compared with those obtained by weighing the fractions eluted from a wider column of the same length as the analytical columns. In the weight analysis the ergosterol has been

included in the calciferol - tachysterol$_2$ fraction, and the analytical figure for tachysterol$_2$ has been corrected for loss during analysis. The differences between the two sets of results for the major fractions are probably due to the presence of small amounts of suprasterols$_2$ I and II. The first fraction (65 per cent. by weight) was converted to the equilibrium mixture by heating in benzene and re-chromatography of this mixture gave a fraction (38 per cent. by weight) containing only calciferol of fairly high purity.

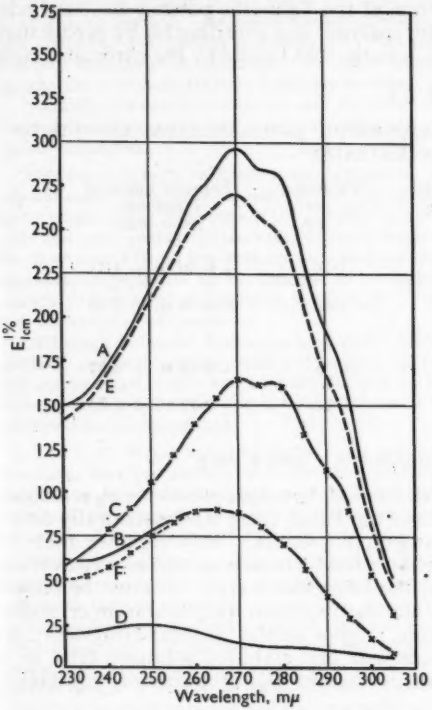

Fig. 1. Absorption spectra: curve A, sample No. 5; curve B, precalciferol$_2$ fraction; curve C, calciferol fraction; curve D, column "strippings"; curve E, sum of B, C and D; curve F, calculated curve for the precalciferol$_2$ fraction

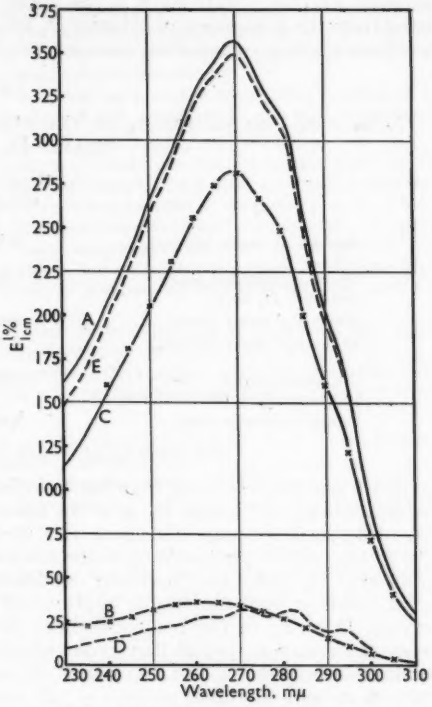

Fig. 2. Absorption spectra: curve A, sample No. 9; curve B, precalciferol$_2$ fraction; curve C, vitamin-D$_2$ fraction; curve D, column "strippings"; curve E, sum of B, C and D

TABLE V

CHROMATOGRAPHIC ANALYSIS OF SAMPLE NO. 12

	Composition found by analysis, %		Fraction weight %
Lumisterol$_2$	11·9	63·7	65·0
Precalciferol$_2$	51·8		
Calciferol	5·7	23·8	24·9
Tachysterol$_2$	16·3*		
Ergosterol	1·8		
Column "strippings"†	—		11·4

* Corrected for loss during analysis.

† Eluted with 25 per cent. v/v of ethanol in acetone.

On suitable materials the methods described here give more accurate results and yield more information than the usual biological assays for vitamins D. Preliminary biological tests on precalciferol$_2$ suggest a potency distinctly lower than that of calciferol; such potency may well be only apparent and the true potency not determinable because of uncertainty about the proportion of calciferol formed during the time required for completion of a biological assay. For this reason, biological results on materials containing a preponderance of precalciferols were considered valueless, and the figures given in Table VI for vitamin-D$_3$ concentrates refer to samples containing only small amounts of precalciferol$_3$. In calculating the chromatographic results given in the upper portion of the Table the potency has been calculated from the percentage of vitamin D$_3$ found by analysis; any contribution by precalciferol$_3$ has been ignored. Statistical examination shows clearly that results by the chromatographic

TABLE VI

EFFECT OF PRECALCIFEROL$_3$ ON THE CHROMATOGRAPHIC AND BIOLOGICAL RESULTS ON VITAMIN-D$_3$ CONCENTRATES*

	Mean result, i.u. per g $\times 10^6$	Difference, i.u. per g $\times 10^6$	Fiducial limits of difference (P = 0·95)
Biological assay (chicks) ..	8·74		
		1·10	+ 0·50 to + 1·71
Chromatographic assay (vitamin D$_3$ only) ..	7·64		
		1·04	+ 0·50 to + 1·59
Biological assay (rats)	8·68		
Biological assay (chicks) ..	8·74		
		0·28	− 0·31 to + 0·87
Chromatographic assay (vitamin D$_3$ + precalciferol$_3$)	8·46		
		0·22	− 0·31 to + 0·75
Biological assay (rats) ..	8·68		

* Nineteen samples, each approximately 8×10^6 i.u. per g.

assay are significantly lower than the biological results. If, however, precalciferol$_3$ is assigned the full potency of vitamin D$_3$, as in the lower half of the Table, there is no statistically detectable difference between biological and chromatographic results. This suggests that the 5 to 10 per cent. of precalciferol$_3$ in the samples under consideration is as biologically available as vitamin D$_3$ under the particular conditions of the biological assays. It must be remembered, however, that the sample solution and the standard solution (prepared from crystalline vitamin D$_3$) used in the biological assay normally receive similar thermal treatment; any small differences in precalciferol contents between test and standard solutions tend to be eliminated because of the formation of a similar vitamin D$_3$ - precalciferol$_3$ equilibrium mixture in both solutions.

We express our thanks to Dr. W. F. J. Cuthbertson and Mr. B. Basil, for carrying out the biological assays and the statistical examination of the results, to Mr. T. E. Holt and Dr. J. C. Clayton for preparing many of the samples and to Mr. J. L. Holmes, Mr. R. L. Smith and Mr. H. T. Brookes for assistance with the practical work.

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NOTE—Reference 1 is to Part I of this series.

GLAXO LABORATORIES LTD.
GREENFORD, MIDDX.

June 7th, 1956

DISCUSSION

DR. G. E. FOSTER, stating that some of the products resulting from the irradiation of ergosterol were photosensitive and, on that account, might undergo change during the determination of their ultra-violet absorption spectra, wished to know if it had been found necessary to take special precautions to avoid errors due to this change.

MR. SHAW replied that while it was perfectly true that, with the exception of suprasterols I and II, all the compounds in the vitamin-D irradiation series were affected by ultra-violet radiation, the amounts of energy required to produce any appreciable photochemical change were considerable. If a photographic spectrophotometer were used, flow-through cells were essential to obtain satisfactory results. On the other hand, the monochromatic radiation passing through solutions during the normal measurement of extinction values in a photo-electric spectrophotometer was much too weak to cause any detectable change, even if the time taken over the measurements was unduly prolonged.

DR. J. H. HAMENCE thanked the authors for bringing a very important matter before the Society, and pointed out that vitamin D was one of the most important vitamins for which a satisfactory chemical test had still to be devised. He asked the authors why it was that the antimony trichloride test broke down when applied to poor-quality concentrates. This was his own experience in applying the test to the determination of vitamin D in foodstuffs. He asked whether the nature of the interfering substance was known and whether any methods were available to overcome or eliminate this interference.

Secondly, he enquired whether any indicator dyes that would indicate the appropriate fraction to be collected for subsequent testing by the antimony trichloride test were available for use in the chromatographic separation.

MR. SHAW in reply said that many substances, particularly those of a steroid nature, gave weak colours with antimony trichloride reagent; this colour reaction, however, remained the most generally satisfactory one for small amounts of vitamin D. Interfering substances, including vitamin A, were often present in foodstuffs and poor-quality concentrates in much larger amounts than vitamin D, and the quantitative separation of vitamin D from low-potency materials remained a major analytical problem. The method described was intended primarily for the analysis of irradiation products, but could also be applied to suitable high-potency preparations of synthetic vitamin D. The analysis of low-potency materials had been excluded from the scope of the work.

Although indicator dyes were used in the standardisation of the adsorbent, they had not been found necessary in the chromatographic separation; the antimony trichloride reaction applied to about 0·05 ml of each fraction had been found very satisfactory for the detection of the positions of the two main bands and it was considered that the use of dyes might lead to difficulties in the subsequent spectrophotometric and colorimetric determinations.

DR. E. C. WOOD enquired if there was any evidence, other than that of the authors' experiments as described, that precalciferol had the same biological activity as calciferol. The agreement between the chemical assay and the bioassay might still be statistically satisfactory if the precalciferol were assumed to have half, or twice, the activity of calciferol, since the proportion of the former was relatively small and the limits of biological error were relatively large.

MR. SHAW said the question of the biological activity of precalciferol was an exceedingly interesting one. It must be remembered that samples containing a 5 to 10 per cent. proportion of precalciferol were being compared with a standard preparation, which probably contained similar amounts. The satisfactory agreement between the biological and chemical results obtained by including the precalciferol in calculating the potency did not necessarily mean that precalciferol had the same biological activity as vitamin D. An attempt had been made to determine the activity of precalciferol$_2$ by administering it to test animals in a single dose. A freshly prepared solution of precalciferol in arachis oil was given in this way at a suitable level to a group of rats and the response compared with that of a similar group receiving calciferol at an appropriate lower dose-level. Under these conditions precalciferol$_2$ had an apparent activity about two-fifths of that of calciferol. No great reliance should be placed on the result at this stage, as no detailed investigation had been made. If the result could be accepted as correct, it did not necessarily mean that this was the activity of precalciferol. Two other explanations were possible (*i*) that precalciferol had no antirachitic activity, the apparent activity representing the amount of change by thermal equilibration to calciferol during the period required for completion of the assay, or (*ii*) that precalciferol was as active as calciferol, but was less fully absorbed by the test animals. At present no satisfactory method was available for the further investigation of this question.

MR. K. A. PROCTOR asked if the authors would define more precisely their "moderately active alumina"; he would like to know how closely this activity must be controlled in order to reproduce their elution series from experiment to experiment.

MR. SHAW answered that the activity of the alumina used was approximately Brockmann grade III. Slight variations in activity were permissible and if desired these could be compensated for by raising or lowering the percentage of acetone in the eluting solvent. No attempt had been made to give the full practical details of the method at the meeting.

MR. E. A. ELSBURY asked if the authors had repeated Dr. Ewing's work on the separation of vitamin A from vitamin D by preliminary passage through a column of Superfiltrol.

MR. SHAW said that the authors were aware of Dr. Ewing's work on the separation of vitamins A and D, but this separation had not been studied in the course of the work described. Superfiltrol and Floridin earth had both been tried with irradiation products, but the main difficulty encountered was in securing reproducible activity of the adsorbent, and for this reason aluminium oxide was preferable.

DR. A. L. GLENN asked if it would be possible to avoid the chromatographic separation by making use of the extinction shift that would occur on changing the temperature of the mixture; the lower potential accuracy of such a method might be offset by its simplicity and speed.

MR. SHAW replied that the increase in extinction value occurring during the thermal transformation of precalciferol into calciferol could be used to follow the change in simple mixtures of the two, and the change during equilibriation might also form the basis of an approximate method for determining precalciferol in irradiation products. Any calciferol already present in the sample would not be determined; and for a detailed analysis preliminary chromatographic separation was essential.

MR. G. I. SMALES enquired if partition chromatography had been tried by the authors.

MR. SHAW said in reply that partition chromatography had not been used for this.

The Determination of Traces of Boron in Nickel

BY R. C. CHIRNSIDE, H. J. CLULEY AND P. M. C. PROFFITT

It was required to determine boron at the level of a few parts per million in nickel sheet used for the anodes of thermionic valves. In the method evolved, the sample is made the anode of a mercury-cathode electrolysis apparatus and dissolution of the sample and deposition of dissolved nickel into the mercury proceed simultaneously; the boron in the electrolyte is then determined spectrophotometrically by the curcumin method. The electrolytic technique is advantageous in effecting solution of the sample and removal of the nickel by one operation and in minimising the quantities of reagents introduced.

Boron contents from 2 to 23 p.p.m. have been found in the samples examined. By dissolving away only a surface portion of the samples, it has been possible to show that a concentration of boron occurs at the surface of the nickel.

IT has recently been found that the presence of traces of boron in nickel used for anodes can lead to low emission in certain types of thermionic valve. During the course of this work, it was required to determine the boron content of various batches of anode nickel and to investigate the distribution of the boron in the nickel. This paper describes the analytical method developed for this purpose.

Spectrographic examination of the nickel samples showed that the boron present was of the order of a few parts per million. Familiarity with the spectrophotometric determination of boron with curcumin, coupled with its very high sensitivity, made the curcumin method an obvious choice for the final determination of the boron. The main problem in developing the required analytical method lay in the procedure to be adopted for removal of the nickel before the determination of the boron.

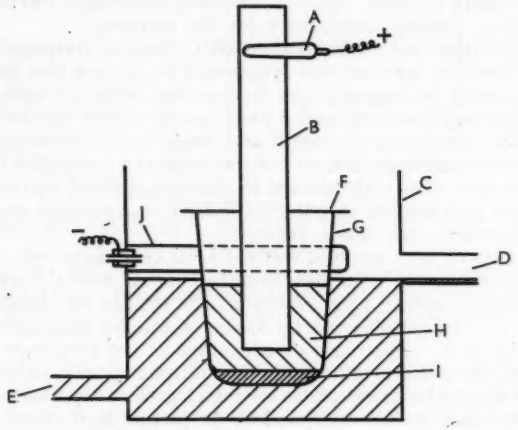
EXPERIMENTAL

REMOVAL OF NICKEL BEFORE DETERMINATION OF BORON—

In the determination of boron with curcumin, it is necessary to make the aqueous boron solution alkaline and then evaporate to dryness before developing the curcumin colour. However, large amounts of alkali salts are deleterious; Silverman and Trego,[1] who use sodium carbonate to render the boron solution alkaline, state that not more than 0·1 g of the carbonate should be used for this purpose. In order to apply the curcumin method under favourable conditions, it is necessary to restrict the amount of acid present in the solution. An undesirable excess of acid can not be eliminated by evaporation, as loss of boric acid by volatilisation would occur. It was therefore required to effect the solution of the sample and the subsequent removal of the nickel by a procedure that introduced the minimum quantities of acid.

For removal of nickel from solution, electrolysis over a mercury cathode appeared to be the simplest method; traces of certain other metals known to be present in the nickel, *e.g.*, iron and manganese, would also be removed by this method. However, electrolysis in the normal manner from a sulphuric acid solution, for example after dissolving the sample

in sulphuric acid - hydrogen peroxide solution, would result in the presence of far more sulphuric acid than appeared desirable for the subsequent application of the curcumin method. This difficulty was overcome by dissolving the nickel sample electrolytically in sulphuric acid solution and simultaneously removing the dissolved nickel electrolytically, a procedure readily effected by making the sample the anode of a mercury-cathode electrolysis cell. In this manner, acid consumed by the solution of the nickel was regenerated on deposition of the dissolved nickel into the mercury and it was thus possible to use in the electrolyte a quantity of sulphuric acid very much smaller than that equivalent to the total weight of nickel dissolved. Experiments showed that it was practicable to dissolve 0·5 g of nickel in about 30 minutes, the electrolyte being 15 ml of water to which only 0·3 ml of *N* sulphuric acid had been added. By prolonging the electrolysis, much greater quantities of nickel could be handled if required, without the need for any increase in the amount of reagents.



A = crocodile clip for positive lead
B = nickel strip
C = cooling vessel
D = outlet for cooling water
E = inlet for cooling water
F = polythene cover
G = platinum crucible
H = electrolyte
I = mercury
J = spring clip bolted to cooling vessel with external connection to negative lead

Fig. 1. Electrolysis apparatus for dissolving the sample and removing the nickel from solution

A diagram of the electrolysis apparatus is shown in Fig. 1. The electrolysis was carried out in a 25-ml platinum crucible, containing 2 ml of mercury and, as electrolyte, 15 ml of water and 0·3 ml of *N* sulphuric acid. The crucible was fixed in a cold-water bath, the electrolyte being kept cool during the electrolysis to avoid any volatilisation of boric acid. The sample strip was inserted so that the required weight of sample was immersed in the electrolyte. On electrolysis with the sample as anode, the immersed portion of the sample dissolved fairly uniformly, but a slightly preferential attack could be observed at the surface of the electrolyte. This effect ultimately caused fracture of the sample strip at the electrolyte surface, leaving a few small fragments of nickel in the electrolyte. Continuation of the electrolysis with a platinum anode resulted in solution of the fragments, after which the electrolysis was continued for a further 15 minutes to ensure complete deposition of dissolved nickel into the mercury. The procedure always yielded a clear solution, with no visible trace of insoluble matter.

Electrolysis in the manner described, followed by application of the curcumin method to the nickel-free electrolyte, permitted the "over-all" boron content of the nickel strip to be determined. However, the electrolytic technique also afforded a readily controllable method for dissolving away an outer portion of the nickel strip; by this means the distribution of boron through the thickness of the strip could be ascertained. As is shown later, it was found that much of the boron present in the nickel strip was concentrated near the surface.

DETERMINATION OF BORON IN THE ELECTROLYSED SOLUTION—

The development of the boron - curcumin colour is usually effected by evaporation of a solution containing boron, curcumin, oxalic acid, hydrochloric acid, water and an organic solvent (alcohol or acetone), but the experimental conditions advocated in the literature[1,2,3,4,5] differ widely. These differences include the temperature and time of heating, the proportions and manner of addition of the reagents, and the nature of the organic solvent used. For the determination of boron in nickel no attempt was made to investigate all the possible variations of the curcumin method, but the following procedure, developed in other work on boron determination, proved satisfactory for the purpose.

The electrolysed solution was made alkaline with a reagent containing sodium hydroxide and glycerol and the resulting solution was evaporated to dryness in a platinum dish. After gentle ignition to eliminate the glycerol, the residue was extracted with water, the solution was neutralised with hydrochloric acid and a fixed excess of acid was added. After addition of an alcoholic solution containing curcumin and oxalic acid, the mixture was evaporated to dryness by floating the platinum dish on a water bath at a controlled temperature (58° C). The residue was then extracted with alcohol to dissolve the red curcumin derivative and, after being centrifuged and diluted to a known volume, the solution was measured spectrophotometrically to determine the boron present.

The main advantage of the curcumin method is its extremely high sensitivity. Hence, in the procedure outlined above, if the final volume is 50 ml and a 1-cm cell is used for the spectrophotometric measurement, 1 μg of boron can readily be determined; if required, much greater sensitivity can be achieved by having a smaller final volume and by using a cell with a longer light path. The main disadvantage of the curcumin method is that the intensity of the developed colour is affected by a multiplicity of variables. The conversion of curcumin to the red complex takes place only during the evaporation of the boron - curcumin solution and the final colour is dependent on factors that affect the rate of heating and evaporation, amongst which are the temperature of the water bath, the size of the platinum dish, the quantity of salts present and the proportion of alcohol and water in the solution. Nevertheless, rigorous standardisation of the procedure can give adequate accuracy for the determination of a few micrograms of boron.

TABLE I

CALIBRATION RESULTS FOR THE DETERMINATION OF BORON WITH CURCUMIN

Boron present, μg	Absorption at 550 mμ		
	As measured,	Corrected for blank	Corrected absorption per μg of boron
0 (blank)	0·068	—	—
1	0·171	0·103	0·103
3	0·383	0·315	0·105
5	0·561	0·493	0·099

Wavelengths of 535 to 550 mμ have been advocated for the spectrophotometric measurement of the boron - curcumin colour. With the method outlined above, maximum absorption was found to occur at 550 mμ, as shown by the absorption curves in Fig. 2. Absorption of the curcumin reagent solution is negligible at this wavelength, but it is always necessary to make a blank correction to allow for traces of boron in the reagents used. Measurement at 550 mμ was found to give a linear relationship between absorption and boron concentration. Typical calibration results are shown in Table I.

METHOD

APPARATUS—

The electrolysis is carried out in a 25-ml platinum crucible containing mercury to act as the cathode (see Fig. 1). The crucible is held by a spring clip in a small vessel through

which water can be passed to cool the crucible and contents during electrolysis. The crucible is provided with a thin polythene disc to act as cover, the disc having a slit to permit insertion of the nickel strip. The electrical connections are made to the upper part of the nickel strip (positive) and to the platinum crucible via the spring clip (negative). A 110-volt d.c. supply is used in conjunction with a variable resistance to give a current of 3 amps. for the electrolysis.

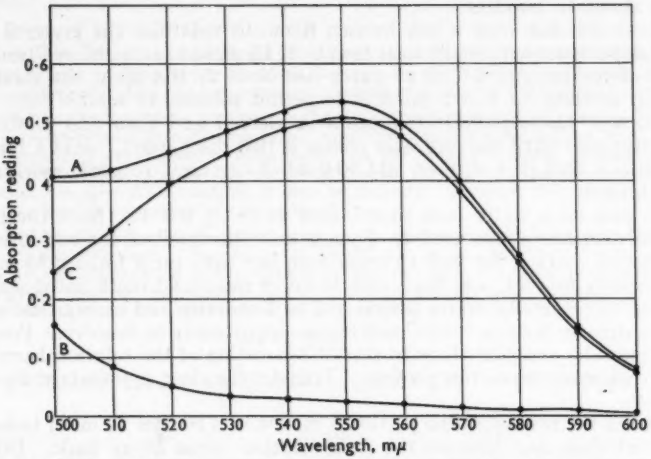


Fig. 2. Absorption spectra of the boron - curcumin colour: curve A, absorption of a standard containing 5 μg of boron; curve B, absorption of blank; curve C, difference curve [A—B] showing net absorption due to 5 μg of boron

A thermostatically controlled water bath, operating at 58° ± 0·3° C, is required for the development of the curcumin colour. It is advantageous to construct a "cage" across the bath by using wire covered with poly(vinyl chloride), so that the floating platinum dish is confined to a particular part of the bath. As variations in the evaporation rate of boron - curcumin solution can affect the development of the colour, the water bath should be in a relatively draught-free room. The platinum dishes used for the colour development procedure should be as nearly identical as possible, as variations in the intensity of the developed colour can arise from the use of dishes of different sizes.

Owing to the very small amount of boron involved, pipettes and other glassware used should not be of the borosilicate type.

REAGENTS—

Standard boron solution A—Dissolve 0·5715 g of boric acid in water and dilute to 1 litre.

1 ml ≡ 100 μg of boron.

Standard boron solution B—Prepare immediately before required by 100-fold dilution of an aliquot of boron solution A, so that

1 ml ≡ 1 μg of boron.

Sodium hydroxide - glycerol reagent—In a polythene beaker, dissolve 2 g of sodium hydroxide and 6 ml of glycerol in water, and dilute with water to 200 ml. Store the solution in a stoppered polythene bottle.

Curcumin reagent—Dissolve 5·0 g of oxalic acid dihydrate and 0·10 g of curcumin in about 400 ml of industrial methylated spirit (74 O.P.). Remove any insoluble matter by filtration, using a Whatman No. 540 filter-paper, and then dilute the filtrate to 500 ml with industrial methylated spirit (74 O.P.). Before being used for filtering the reagent solution, the filter-paper should be extracted for about 30 minutes with 50 ml of hot industrial methylated spirit containing 2 or 3 drops of hydrochloric acid, in order to remove any extractable boron.

PROCEDURE FOR PREPARING THE CALIBRATION GRAPH—

Apply the following procedure separately to 0, 1, 3 and 5-μg amounts of boron.

Add the appropriate volume of standard boron solution B (1 ml $\equiv$ 1 μg of boron) to a platinum dish (a dish having a diameter of 3 inches and a capacity of 75 ml is suitable). Add 0·3 ml of *N* sulphuric acid and 2·0 ml of sodium hydroxide - glycerol reagent, and evaporate the solution to dryness on a steam-bath. Heat the dish and contents in an air-oven at 110° C for about 15 minutes.

Gently heat the dish over a low bunsen flame to volatilise the glycerol present, then increase the temperature and finally heat for about 15 seconds at a dull red heat (see Note 1).

To the cool residue add 2·0 ml of water (see Note 2) and allow the mixture to stand, with occasional swirling for 2 or 3 minutes to permit solution of soluble salts. Add 1 drop of 1 per cent. w/v phenolphthalein solution in alcohol and then add dilute hydrochloric acid (1 + 4) dropwise until the indicator colour is just discharged. Add a further 0·5 ml of dilute hydrochloric acid (1 + 4) then add 10·0 ml of curcumin reagent, swirling the solution during the addition.

Float the dish on a water bath maintained at 58° $\pm$ 0·3° C. Note the time when the solution in the dish has evaporated to dryness and the smell of hydrochloric acid can no longer be detected. Allow the dish to remain on the bath for a further 15 minutes.

Allow the dish to cool, and then add 10 ml of industrial methylated spirit (74 O.P.). Stir the mixture occasionally over a period of 2 or 3 minutes and ensure that any of the red curcumin derivative on the sides of the dish has an opportunity to dissolve. Pour the solution into a centrifuge tube and centrifuge to permit separation of the solution from the insoluble salts and any carbonaceous matter present. Transfer the clear supernatant liquid to a 50-ml calibrated flask.

Again extract the residue in the platinum dish with a further 10 ml of industrial methylated spirit, centrifuge and transfer the clear solution to the 50-ml flask. Dilute the combined extract to the mark with industrial methylated spirit.

Using 1-cm cells, measure the absorption of the solution at 550 mμ, using industrial methylated spirit as the reference solution. Subtract the absorption reading for the solution to which no boron was added from the readings for the other solutions and prepare a calibration graph from the results. The relationship between corrected absorption reading and μg of boron added should be linear.

PROCEDURE—

The sample of nickel strip should preferably be about $\frac{1}{2}$ to $\frac{3}{4}$ inch wide and not less than 3 inches long. The strip should be bent along its length to give a right-angle section to provide additional rigidity.

To the platinum crucible to be used for the electrolysis add 2 ml of mercury, 15 ml of water and 0·3 ml of *N* sulphuric acid. Weigh the sample of nickel strip and then insert it through the slit in the polythene cover into the solution in the platinum crucible; the position of the strip should be such that 0·2 to 0·5 g of the strip is immersed in the solution. (For strip 0·005 inch thick, these weights correspond to immersed areas of 0·3 to 0·7 sq. inches, as measured on one side of the strip.)

Electrolyse at a current of 3 amps. During the electrolysis run a slow stream of water through the cooling bath surrounding the platinum crucible so that the temperature of the cooling water, measured near the crucible, does not exceed 30° C. As the electrolysis proceeds, preferential attack on the immersed nickel takes place at the surface of the electrolyte and ultimately the immersed strip breaks off at this point. Switch off the current immediately this breaking-off occurs. Withdraw the sample of nickel strip, rinse it, dry it and re-weigh it to determine the weight of sample removed (see Note 3).

Insert into the electrolyte through the polythene cover a piece of platinum strip to serve as anode. Continue the electrolysis to effect solution and subsequent deposition into the mercury of any fragments of nickel remaining from the fracture of the sample strip. Continue the electrolysis for 15 minutes after the fragments of metallic nickel can no longer be observed.

Stop the electrolysis and by means of a pipette transfer the electrolyte to a platinum dish reserved for the colour development procedure. Wash the crucible, anode and mercury once with a few millilitres of water and add the washings to the platinum dish.

To the solution in the platinum dish add 2·0 ml of sodium hydroxide - glycerol reagent, evaporate to dryness and proceed with the development and measurement of the curcumin colour exactly as carried out for calibration.

Make a blank determination with the quantities of reagents used in the procedure, but omit the electrolysis. Correct the absorption of the solution derived from the nickel sample by subtracting the absorption of the blank and hence calculate the boron content of the sample.

NOTES—

1. The heating should be carried out in such a manner as to produce the minimum amount of carbonaceous residue, as this tends to retain boron. As much as possible of the glycerol should therefore be driven off before the temperature at which carbonisation takes place is reached.

2. To obtain the maximum development of the curcumin colour it is essential to have a certain proportion of water present when the mixture is evaporated on the water bath at 58° C. The quantity of water to be added will depend to some extent on the amount of water present in the industrial methylated spirit used to prepare the curcumin reagent. Although the recommended addition of 2·0 ml of water has proved satisfactory, it may be desirable to modify this quantity if the expected sensitivity of colour development is not achieved.

3. Electrolytic solution of the nickel strip in the manner described permits the determination of the over-all boron content of the strip. If desired, the boron content of the surface layer can be determined by electrolysing for a few minutes to dissolve only the outer portion of the immersed strip. It is still, however, necessary to continue the electrolysis for 15 minutes with the platinum anode to ensure that all the dissolved nickel is deposited in the mercury.

TABLE II

DETERMINATIONS OF BORON IN SAMPLES OF ANODE NICKEL

Sample No.	Boron found, p.p.m.	Mean, p.p.m.
"Good" anode nickels—		
1	2, 2	2
67	3, 3	3
78	3, 3	3
"Bad" anode nickels—		
5	12, 13	13
16	16, 16, 18	17
161	19, 26	23

TABLE III

DETERMINATION OF THE DISTRIBUTION OF BORON THROUGH NICKEL STRIP

	Surface portion		Residual portion		Over-all boron content	
Sample No.	Part by weight of whole, % (X)	Boron found, p.p.m. (x)	Part by weight of whole, % (Y)	Boron found, p.p.m. (y)	Calculated $\left(\frac{Xx + Yy}{100}\right)$, p.p.m.	Directly determined, p.p.m.
161	35	46	65	8	21	23
	9	160	81	7	20	
	3·2	400	96·8	8	21	
78	9·6	18	90·4	2	3·5	3
	0·6	~90	99·4	2	2·5	

RESULTS AND DISCUSSION

The method devised was applied to different batches of anode nickel, classified as "good" or "bad," according to their influence on the thermionic emission in certain types of valve. A series of results is shown in Table II. This work showed that the boron content was appreciably higher in the "bad" batches of nickel.

Two of the samples referred to in Table II, Nos. 78 and 161, were selected for investigation of the distribution of boron in the nickel. The electrolytic dissolution of each sample was carried out for a few minutes, thus removing only the surface portion; the determination of the boron in the electrolyte was then completed in the usual manner. The sample strip was then immersed to the same depth in fresh electrolyte in the electrolysis apparatus and the immersed portion was completely dissolved electrolytically; this permitted determination of the boron content of the residual section remaining after solution of the surface portion.

As shown in Table III, the results obtained in this manner established that a large portion of the boron present was concentrated at the surface of the strip. Similar results were obtained on other samples.

From the results obtained on the surface and residual portions of a sample examined in this manner, it was possible to calculate the over-all boron content of the sample. As is shown in Table III, the calculated results were in good agreement with the values for over-all boron content obtained by direct determination.

The electrolytic technique used in the method is very convenient in that in one operation it permits dissolution of the sample and preparation of a nickel-free solution suitable for direct application of the curcumin method for boron. The electrolytic procedure is also advantageous in that the only reagents used are 15 ml of water and 0·3 ml of *N* sulphuric acid and this economy of reagents enables low blanks (less than 1 μg of boron) to be obtained. If desired, much larger quantities of nickel could be dissolved and removed from solution by electrolysis without any increase in the quantity of reagents required.

The possibility of loss of boron by volatilisation during the electrolysis has not been specifically investigated for the purpose of the present method. Experience in these laboratories with the determination of percentage amounts of boron in alloys has shown that no loss of boron occurs during a mercury cathode electrolysis, provided that the electrolyte is cooled. This experience is also confirmed by Bush and Higgs,[6] who state that the temperature of the electrolyte can be as high as 65° C without significant loss of boron occurring. It seems reasonable to conclude that, in the method now recommended for traces of boron in nickel, no significant volatilisation of boron occurs during the electrolysis. This is supported by the good agreement between the calculated and directly determined over-all boron contents shown in Table III, particularly as the series of results reported involved electrolyses over widely differing periods of time.

Although the procedure was devised for the particular task of determining traces of boron in nickel, it may also prove to be applicable with advantage to metals other than nickel, provided that the main constituents of the sample can be removed from solution by electrolysis over a mercury cathode.

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Volumetric Determination of Cyanide and Nickel with Resacetophenone Oxime as an Indicator

By K. S. BHATKI

With resacetophenone oxime as an internal indicator, a solution of cyanide can be standardised against a standard nickel solution prepared from extra-pure nickel ammonium sulphate. It has been shown that in a similar manner an indirect determination of nickel can be made, thus avoiding the use of a more expensive reagent such as silver nitrate.

All the methods[1,2] available at present for the determination of cyanide involve the use of silver nitrate, except for one in which a standard nickel solution is used, together with dimethylglyoxime as an external indicator.[3] Silver nitrate is an expensive reagent, whereas the other method involves the inconvenience of an external indicator.

This paper describes the determination of cyanide with a standard nickel solution, resacetophenone oxime being used as an internal indicator. The method has been found to be more accurate and convenient than the previous ones.

Method

Reagents—

Nickel ammonium sulphate solution—Prepared from extra-pure quality reagent; this was the standard nickel solution. The determination of the nickel content of this solution both by the dimethylglyoxime method and by the resacetophenone oxime method[4] showed that the prepared solution was 0·105 *N*.

Nickel chloride solution—This was prepared from the AnalaR reagent and was standardised in the same manner as the nickel ammonium sulphate solution. A suitable solution was found to be 0·161 *N*.

Potassium cyanide solution—Prepared from Merck's 98 to 100 per cent. pure potassium cyanide. This was checked against a standard silver nitrate solution and was found to be 0·144 *N*.

Indicator solution—Prepared by dissolving 2 g of resacetophenone oxime in 100 ml of 40 per cent. ethanol.

Procedure—

Measure out a known volume of potassium cyanide from a burette into a conical flask and dilute to about 75 ml. Add about 10 g of sodium acetate (Merck's extra pure was used) to it and titrate the solution against standard nickel ammonium sulphate, using about five to ten drops of the indicator solution. The end-point is marked by the development of a greenish-yellow precipitate or turbidity.

The reaction taking place can be represented as follows—

$$NiSO_4 + 4KCN = K_2[Ni(CN)_4] + K_2SO_4.$$

The excess of nickel over that required to react with the potassium cyanide then interacts with the resacetophenone oxime and the precipitate or turbidity formed indicates the end-point—

$$NiSO_4 + 2C_8H_9O_3N = (C_8H_8O_3N)_2Ni + H_2SO_4.$$

Nickel chloride is as satisfactory a titrant as the nickel ammonium sulphate, as seen from the results shown in Table I.

Table I

Results by direct method

10 ml of KCN ≡ 13·66 ml of nickel ammonium sulphate
10 ml of KCN ≡ 8·92 ml of nickel chloride

Volume of potassium cyanide taken, ml	Volume of nickel ammonium sulphate		Volume of nickel chloride	
	Calculated, ml	Used, ml	Calculated, ml	Used, ml
15·00	20·49	20·40	13·38	13·45
17·00	23·21	23·10	15·16	15·20
20·00	27·32	27·20	17·83	17·80
22·00	30·04	29·95	19·62	19·65
25·00	34·14	34·00	22·29	22·25
27·00	36·38	36·75	24·08	24·00
30·00	40·98	40·80	26·75	26·80
32·00	43·70	43·60	28·54	28·55
35·00	47·80	47·75	31·22	31·25

The determination of nickel can also be made by an indirect method as follows.

By pipette put a known volume of the nickel solution into a conical flask. Add dilute sodium carbonate solution until a slight turbidity is observed. Add dilute acetic acid until the solution is just clear and then 10 g of sodium acetate. Treat the resulting solution with an excess of the standard potassium cyanide solution, added from a burette, so that the precipitate formed is redissolved and a clear solution obtained. Determine the excess of potassium cyanide by titrating against a standard nickel solution.

Typical results by this method are shown in Table II.

TABLE II

RESULTS BY INDIRECT METHOD

10 ml of KCN ≡ 13·66 ml of nickel ammonium sulphate
10 ml of KCN ≡ 8·92 ml of nickel chloride

Volume of nickel chloride taken, ml	Amount of nickel chloride present, mg	Volume of potassium cyanide added, ml	Volume of nickel ammonium sulphate required for excess of potassium cyanide, ml	Amount of nickel chloride found, mg
10·00	191·0	21·00	13·35	190·6
10·00	191·0	12·00	1·15	190·2
15·00	286·6	20·00	4·40	285·9
20·00	382·1	23·00	0·80	381·9
20·00	382·1	32·50	13·75	381·9
23·00	439·4	30·00	5·80	438·7
25·00	477·6	30·00	2·75	476·9
30·00	573·1	35·00	1·80	574·0
30·00	573·1	43·70	13·65	573·8

The addition of sodium acetate is necessary, because nickel - resacetophenone oxime, which indicates the end-point, is formed at pH values above 6·2 (but below 11·0). For ensuring the correct pH the addition of about 10 g of sodium acetate was found to be satisfactory.

Copper, cobalt, zinc and certain other metals that generally occur with nickel are known to form complex cyanides and hence nickel cannot be determined by this method in their presence.

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The Determination of Mercury in Air

By G. A. SERGEANT, B. E. DIXON and R. G. LIDZEY

A method has been developed for the determination of all forms of mercury in air at concentrations in the neighbourhood of 100 μg per cubic metre. The vapour of mercury and its compounds is taken up by iodised active carbon, and mercury-bearing dust is trapped in a mineral-wool filter. On heating in a specially constructed ignition tube, the mercury is transferred as vapour to a selenium sulphide test paper, giving a stain that is compared with a set of standards representing known concentrations of mercury in air for a given volume of sample.

The work described in this paper was carried out with the object of developing a reliable and relatively simple, although not necessarily very precise, method for determining all forms of mercury, *i.e.*, mercury or mercury compounds present as vapour, spray or dust, in concentrations around the usually accepted "toxic limit" of 100 μg per cubic metre in industrial and laboratory atmospheres. Elaborate apparatus and unstable reagents were to be avoided, and the samples required were to be as small as possible to avoid the use of heavy pumping equipment.

A review of the literature indicated that no existing method for mercury in air could be readily adapted, but that described by Nordlander[1] appeared to provide a possible basis for development.

The Nordlander selenium sulphide method—

When hydrogen sulphide is passed into selenous acid solution, a yellow precipitate is formed in accordance with the following equation—

$$H_2SeO_3 + 2H_2S = 3H_2O + Se + 2S.$$

This "selenium sulphide" probably consists neither of a compound nor a simple mixture of selenium and sulphur, but of finely divided mixed crystals. It was observed by Nordlander that this, and other sulphur - selenium solids, darken in contact with mercury vapour and provide a means of detecting mercury vapour that is specific on account of the relative non-volatility of the other metals that give rise to dark-coloured sulphides and selenides.

The preparation of precipitated selenium sulphide suitable for coating test paper is described by Nordlander, and precipitation direct on filter-paper has also been used.[2] A modified method of preparing test paper is described later.

In the main forms of Nordlander mercury-vapour detector the air to be tested for mercury is heated (to increase sensitivity) and is then projected either by means of a pump[1] or by thermal convection[3] against a paper surface coated with selenium sulphide. Under these conditions the sorption of mercury on the sensitive surface is very inefficient, so that sensitivity is low. It has been found that only at a rate of airflow too slow for a practical sampling procedure can mercury vapour be quantitatively recovered from air by passage through filter-paper impregnated with selenium sulphide, so that mercury vapour must first be collected by some other means if the advantages of selenium sulphide as a detector are to be realised in practice. In the present work iodised active carbon is used for trapping mercury vapour.

Active carbon as a sorbent for mercury vapour—

Untreated active carbons are generally inefficient sorbents for mercury vapour, and iodine-impregnated active carbon was proposed by Stock.[4] Active carbon containing 5 per cent. w/w of iodine is used in canister respirators intended for protection against mercury vapour.[5] For analytical purposes a high iodine content in the carbon complicates the subsequent recovery of traces of trapped mercury; however, it has been found that, when a high total capacity is not required, small additions of iodine to active carbon greatly increase its sorbent efficiency for microgram quantities of mercury vapour. The mercury held in the carbon may be released by mild ignition. In the presence of iron powder any liberated iodine is retained and any mercury iodide decomposed.

PARTICULATE MERCURY AND MERCURY COMPOUNDS PRESENT AS VAPOUR AND DUST—

The vapour of mercury compounds is readily taken up by a suitable grade of active carbon even in the absence of iodine, while particulate mercury and mercury-bearing dust may conveniently be trapped in a filter of mineral-wool such as Stillite filter-wool, which, moreover, is not affected by moderate ignition.

The ignition method is simplified by the fact that most mercury compounds decompose on heating to give mercury, while those that do not, *e.g.*, mercuric chloride, ethylmercury chloride, diethylmercury and so on, are decomposed by heating with an excess of active carbon and iron powder.

EXPERIMENTAL

MERCURY-VAPOUR STANDARD—

Water was boiled in a flask containing also about 1 ml of mercury, and the resulting steam - mercury-vapour mixture was carried by a slow airstream entering the flask (not more than 100 ml per minute) into two reflux condensers connected in series. The upper condenser, which was of the Revenue type with a long condensing spiral, was supplied with water thermostatically controlled at 22° C and the outlet from this ran through the lower condenser. Air emerging from the upper condenser was therefore saturated at 22° C with water and mercury vapour, and by mixing it with pure air, any desired lower concentration of mercury vapour could be obtained.

The output of the generator was checked from time to time by an absorptiometric dithizone method, acid permanganate in a sintered-glass bubbler being used for collecting the mercury vapour, and found to be in reasonable agreement with that calculated from the available vapour-pressure data.

TESTING OF IODISED ACTIVE-CARBON FILTERS—

In each of a series of tests, 50 litres of air containing 10 to 20 μg of mercury were passed at 2½ litres per minute through two sampling tubes (see p. 31) connected in series. Comparison was then made between the respective amounts of mercury collected by the carbon in the first and second tubes, the ignition-tube method of analysis to be described later being used. These tests indicated an efficiency in excess of 90 per cent. for the type of iodised active-carbon filter finally adopted (see Table I).

VOLATILE MERCURY COMPOUNDS—

Mercuric chloride, ethylmercury chloride, diethylmercury, di-*n*-butylmercury, diphenylmercury and di-*p*-tolylmercury were all examined in this connection. In the tests a slow airstream was charged with the vapour of the compound, either at room temperature (diethylmercury and di-*n*-butylmercury) or at 100° C, and this air was mixed with a large excess of pure air to give a concentration of mercury in the region of the "toxic limit." The prepared air - mercury-vapour mixture was passed at 2½ litres per minute through two iodised active-carbon filters connected in series, in the manner of the mercury-vapour tests described above.

For all the compounds examined, trapping efficiencies of around 90 per cent. were observed for the iodised active-carbon filter (Table I).

TABLE I

COLLECTION OF MERCURIAL VAPOUR BY IODISED ACTIVE-CARBON FILTER

Vapour	Mercury content of first filter, μg	Mercury content of second filter, μg	Efficiency of filter, %
Mercury	20	1	95
	20	½	97
	20	0	100
Mercuric chloride	10	1	90
	15	1	93
Ethylmercury chloride ..	40	3	92
Diethylmercury	20	2	90
	20	3	85
Di-*n*-butylmercury	20	2	90
Di-*p*-tolylmercury	5	1	80
	20	2	90
Diphenylmercury	5	1	80
	20	2	90

TABLE II

COLLECTION OF MERCURIAL DUSTS BY MINERAL-WOOL FILTER

Dust	Mercury content of mineral-wool filter, μg	Mercury content of filter effluent (electrostatic precipitator), μg	Efficiency of filter, %
Mercuric chloride	20	3	87
	20	3	87
Mercuric iodide	20	2	91
	15	2	88
Diphenylmercury	30	2	94
	30	4	88
	20	3	87
Di-*p*-tolylmercury	30	5	85
	30	3	91

GENERATION OF MERCURY-BEARING DUST—

It was not found convenient by direct grinding followed by air levigation to produce sufficient dust of the required degree of fineness (1 μ and less), and in the method finally adopted mercury compounds were volatilised and allowed to condense in an airstream, to give a very fine dust-cloud or smoke. Mercuric chloride, mercuric iodide, diphenylmercury and di-*p*-tolylmercury were used in these tests. The mercury compound was heated electrically in a small tube suspended inside a Winchester-quart bottle, and the smoke was driven out by a rapid airstream into a 1-litre mixing flask.

Samples of smokes produced by the generator were collected by an electrostatic sampler and examined under the microscope. A high degree of uniformity was observed, and the average particle size was estimated to be well under 1 μ.

TESTING OF DUST FILTERS—

In each test, an airstream of 2½ litres per minute from the smoke generator was passed through the filter under examination. Solid material passing through the filter was collected by a small electrostatic precipitator,[6] from the glass collecting tube of which it could be recovered by sweeping through with a plug of asbestos fibre. Mercury in the filter and in the asbestos fibre was then determined by the ignition-tube method.

Mineral-wool filters prepared in the manner described later were found in this way to have an efficiency of about 90 per cent. for smoke from the generator (Table II).

METHOD OF PREPARING APPARATUS AND MATERIALS

PREPARATION OF IODISED ACTIVE CARBON—

Direct addition of iodine to active carbon in a stirring machine was tried successfully, but was later abandoned in favour of a high-temperature method of preparation in which the carbon is iodised and at the same time freed from water and any other undesirable volatile impurities. Some iodine is lost in the process in order to ensure the even distribution of that remaining. Although free iodine might appear to be necessary for mercury retention, it is probable that the residual activating iodine is actually present as iodide, by reaction with the alkali content of the carbon. Hence alkali and ammonium iodides may be used to activate the carbon by the ignition process.

The basic material is Active Carbon 207 C, 18 to 30-mesh, obtainable from Sutcliffe, Speakman & Co. Ltd., Leigh, Lancs. Re-sift a quantity of the carbon to remove dust, and weigh out 50 g of the sifted material. Add 1 g of iodoform powder and mix well. Transfer the mixture to a covered porcelain basin or crucible and heat in a furnace to about 800° C. Then, after sufficient cooling, store the prepared carbon in a desiccator.

PREPARATION OF TEST PAPER—

Dip sheets of Whatman No. 1 filter-paper in a 2 per cent. w/v selenous acid solution; allow them to drain for a few minutes and finally dry them at 100° C. Trim about 1 cm off the edges of the dried paper and store the sheets in a stoppered bottle. Prepare each piece of test paper immediately before use by adding one drop of a 5 per cent. w/v thioacetamide

solution in ethanol to a piece of the impregnated paper of roughly postage-stamp size, and allowing the paper to dry for a few minutes while the orange colour develops.

It is desirable always to use the same side of the test paper (the smooth side), and this can be ensured by marking the reverse side of the paper with a pencil before preparation.

THE IGNITION TUBE*—

The ignition tube is designed to enable mercury to be quantitatively transferred from a mercury-bearing charge of active carbon and mineral-wool to a circular area of test paper.

Heating is effected by a selected type of gas burner consuming gas at a given rate. Mercury and mercury compounds are thereby volatilised and are then swept along the tube to the test paper by a stream of carbon monoxide generated by the decomposition of a small charge of sodium oxalate in the tube. The presence of both active carbon and iron powder in the tube ensures that only uncombined mercury reaches the test paper.

For a successful ignition, the test paper must not become overheated and scorched, yet mercury vapour, unless present in excessive amount, must not be lost in transit by condensation in the cooler parts of the tube. Further, the decomposition of the sodium oxalate is required to take place gradually, as a violent evolution of gas would cause incomplete retention of mercury vapour by the test paper. To satisfy these requirements, it was necessary to pay careful attention to the design and materials of construction of the tube and also to the operating conditions.

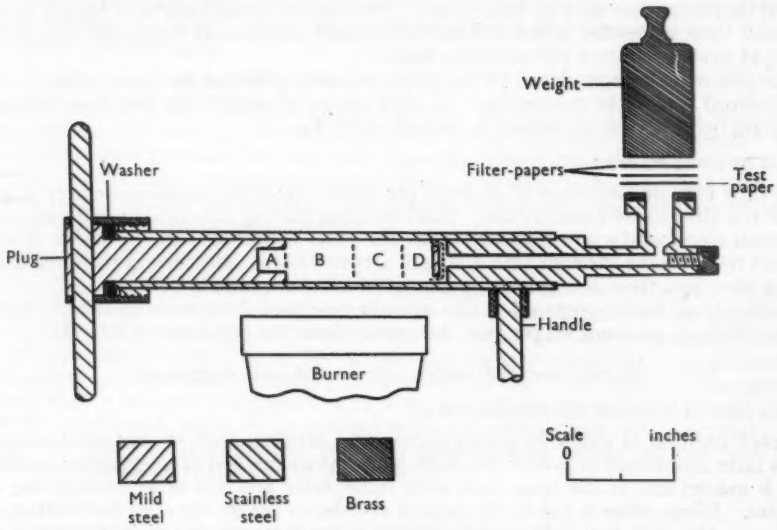

Fig. 1. Vertical section of the charged ignition tube: A, sodium oxalate; B, mineral-wool; C, iodised carbon; D, iron powder

Fig. 1 shows a vertical section of the tube charged ready for an ignition. The construction is mainly of stainless steel (Staybrite F.D.P.), although the screw-in plug is of mild steel, the higher heat conductivity of which is necessary to prevent too rapid a rise in temperature of the sodium oxalate charge contained in the cavity during an ignition. A steel disc, around which is wrapped a piece of fine iron gauze, fits tightly against the end of the capillary tube leading from the main chamber to the head, and prevents any part of the charge from entering the capillary. A gas-tight seal is provided at the plug end of the tube by means of an asbestos compound washer covered by a layer of graphite powder, which is kept "topped up" by occasional additions of the powder. A ring of Paxolin over the head protects the test paper from scorching.

* This and other items of equipment for the test are being made available commercially.

THE SAMPLING TUBE—

The sampling tube is shown in Fig. 2. The inlet side, which consists of a piece of glass tubing (10½ to 11 mm internal diameter) containing the mineral-wool filter, is attached by a short piece of rubber tubing to a central glass bulb (25 mm external diameter) partly filled with granular calcium chloride (3 g of 8 to 14-mesh) held in place by cotton-wool. The narrow outlet tube (5½ to 6 mm internal diameter) holds the active carbon (0·35 g), which is retained by thimbles of fine steel gauze.

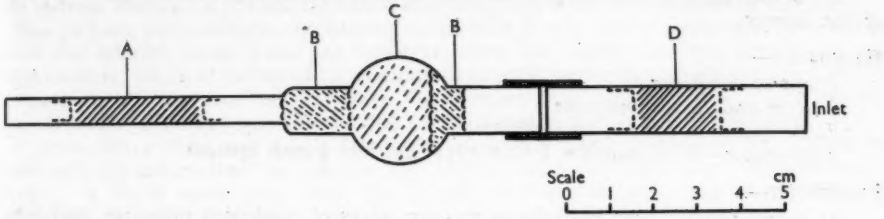

Fig. 2. Sampling tube: A, iodised active carbon; B, cotton-wool; C, calcium chloride; D, mineral-wool filter

The calcium chloride serves to protect the carbon against excessive moisture absorption, especially when sampling under humid conditions, as it has been observed that this may subsequently cause blurring of the stain on the test paper.

To prepare the dust filter, heat a quantity of Stillite filter-wool, standard grade, to 450° to 500° C, and maintain it at this temperature until all carbonaceous matter has burned away. Cool, and weigh out 0·8 g of the ignited material, avoiding patches of "shot" that may be encountered, and insert the weighed portion in the inlet tube, where it is to be moulded into the form of a cylinder 1 cm in diameter and 2 cm long by compression between two retaining thimbles shaped out of open-mesh wire gauze.

Connect the completed inlet and outlet sections by a short piece of rubber tubing, and, if the tube is not to be used at once, seal the inlet end with a rubber bung and the outlet end by means of a short piece of rubber tubing carrying a glass plug.

METHOD OF SAMPLING

APPARATUS—

Pump, or other source of suction, capable, for the maximum sampling rate, of withdrawing 2½ litres of air per minute against a back pressure of 2 to 3 pounds per square inch.

Flowmeter to indicate rate of sampling.

Sampling tube.

Reservoir to contain 1 litre, with inlet and outlet connections.

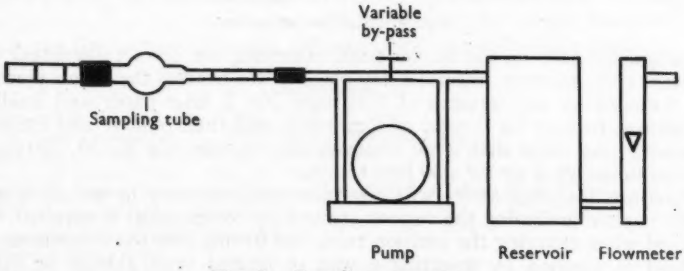

Fig. 3. Sampling apparatus

PROCEDURE—

In the sampling arrangement shown in Fig. 3 the sampling rate may be controlled, as shown, by a screw clip in a by-pass across the pump. If a vacuum line or water-jet pump is used as a source of suction, the flowmeter is connected directly between this and the sampling tube; no reservoir is required, as pulsations in the air flow will be absent or only slight, but the flowmeter is working at reduced pressure and the readings must be corrected accordingly.

Adjust the sampling rate so that 50 litres of the atmosphere being tested are drawn through the sampling tube in not less than 20 minutes.

Method of ignition

Apparatus—

Ignition tube.

Burner—Amal major laboratory bunsen burner fitted with tube rests.

Flowmeter (*e.g.*, a Rotameter) calibrated to indicate the approximate gas consumption of the burner.

Materials—

Prepared test paper.

Sodium oxalate, reagent grade.

Electrolytic iron powder, 40 to 60-mesh.

Filter-paper—Whatman No. 1 filter-paper cut into ¾-inch squares.

Procedure—

As a preliminary, heat the tube to remove traces of condensed moisture, and then, as soon as it can be easily handled, remove the plug and introduce the following items successively—

(*a*) 2 g of iron powder,

(*b*) the iodised carbon from the sampling tube; this must be transferred directly and not be allowed to pick up moisture by undue exposure to the atmosphere, and

(*c*) the mineral-wool filter from the sampling tube, pressed well in.

Fill the cavity in the plug with sodium oxalate, packed in nearly to the brim. To the recess containing the washer add a little graphite powder, should this be necessary to maintain a continuous sealing ring of the powder, and then screw the plug firmly into the tube (Fig. 1).

Adjust the burner to consume about 7 litres of gas per minute. The central cone of the flame should lie flat on the grid of the burner, and some re-setting of the inlet nozzle may be necessary to effect this.

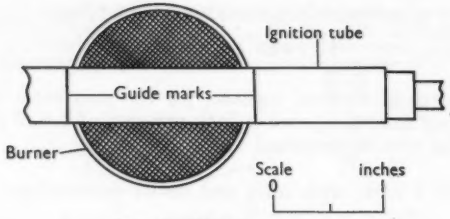

Fig. 4. Position of burner

Lay the ignition tube on the burner rests, observing the correct alignment by means of the guide marks (Fig. 4), then, without delay place on the head of the tube a piece of prepared test paper, followed by two squares of Whatman No. 1 filter-paper and finally the brass weight. Continue to heat for a total of 8 minutes, and then remove and examine the test paper, comparing any stain with a set of standards representing 25, 50, 100 and 200 μg of mercury per cubic metre of air for a 50-litre sample.

The procedure described above will determine total mercury in the air sample. When only mercury vapour (including the vapour of mercury compounds) is required, the mineral-wool is omitted when charging the ignition tube, but in this case the other items in the tube should be held in position by inserting a wad of ignited mineral-wool or asbestos fibre. Similarly if it is required only to estimate mercury in the form of dust, the iodised active carbon from the sampling tube is not tranferred to the ignition tube, but instead a similar quantity of dry unused iodised active carbon is put in to fill the tube.

The stains are permanent, although the background colour gradually becomes deeper. If desired, the background colour may be removed without appreciably affecting the stain. For this purpose, immerse the test paper carrying the stain in 16 per cent. ammonium sulphide solution diluted with water (1 + 3), for 1 to 2 minutes, and then wash the paper thoroughly

in tap water with a final rinse in distilled water. Place the bleached paper on dry filter-paper and complete the drying at not more than 100° C. As a further step, confirmation of the presence of mercury, should this be needed, may be obtained by cutting the paper in half and exposing the two halves to the vapour of bromine and iodine, respectively. Bromine causes immediate bleaching, and iodine a gradual reddening of the stain.

After an ignition giving a stain equal to or in excess of the darkest of the standards, the tube should be "swept out," more than once if necessary, by further ignition with renewal of the sodium oxalate only, until the blank stain obtained is small enough to be neglected.

The latitude permissible in the heating of the tube is sufficient to cover any likely variations in the calorific value of the gas; however, when the heating has been insufficient, the sodium oxalate, which is darkened by decomposition, will be found to have remained white, and, when the heating has been excessive, the mercury stain is liable to be blurred at the edges, although this effect may also be due to the presence of moisture in the charge.

A preliminary blank test should be carried out with a charge of iron powder and sodium oxalate only, to ensure that the ignition tube and materials are free from contamination by mercury. A blank stain paler than the lightest of the standards may be regarded as satisfactory.

Between successive ignitions the ignition tube must be allowed to cool sufficiently to be readily handled. This procedure, which, unassisted, occupies about 20 minutes, may be greatly hastened if a small centrifugal blower is available.

After many ignitions there is a tendency for corrosion products to accumulate in the capillary tube, which should be cleaned occasionally by forcing through it a small wad of steel-wool.

Preparation of standard stains

Standard stains may be obtained by igniting known amounts of mercury or mercury compounds in the ignition tube under the usual operating conditions. From soft porous alumina firebrick, cut a number of small bricks each about 6 mm square by 20 mm long. Ignite the bricks to remove any contamination. Impregnate each brick by adding first one drop of 5 per cent. w/v sodium sulphide solution and then, from a microburette, transfer the appropriate volume of standard mercuric chloride (100 μg mercury per ml) to give the required series of standards up to 10 μg of mercury, above which the stains become too dark for satisfactory matching. Dry the bricks at 100° C for 2 hours and store them in a stoppered steelbottle.

For the degree of accuracy intended, a series of four standard stains representing, respectively, 25, 50, 100 and 200 μg of mercury per cubic metre of air for a 50-litre sample is adequate. It is, however, possible to estimate the intensity of stains towards the upper end of this range to an accuracy of about $\pm$ 10 per cent.

This work was carried out on behalf of the Factory Department, Ministry of Labour and National Service, and the Department of Scientific and Industrial Research.

We thank the Government Chemist for permission to publish this paper.

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June 18th, 1956

The Use of Zincon in the Absorptiometric Determination of Mercury

By A. G. MORRIS

A procedure for the detection and absorptiometric determination of mercuric mercury with use of zincon as a reagent has been developed. The reaction has been studied with particular reference to optimum pH conditions and interference from other ions. Details are given of a procedure covering a working range of about 3 to 8 p.p.m. of mercuric mercury.

Zincon (2-carboxy-2′-hydroxy-5′-sulphoformazylbenzene) has recently received some attention as a colorimetric reagent for zinc and copper.[1] While investigating interferences affecting these procedures, I observed that zincon also gave a stable and sensitive colour reaction with mercuric mercury. In view of the comparative scarcity of good colorimetric reagents for this metal, it was decided to investigate the reaction more fully.

The following account of the work undertaken confirms that the reagent can be used with advantage for the detection and colorimetric determination of mercuric mercury. Mercurous compounds do not yield intense colours with zincon, but the mercuric complex is intensely blue and is stable in solution for at least 2 hours. The wavelength - absorbance curve exhibits an absorption plateau in the region of 600 mμ and the optical density near this wavelength varies with pH, showing a maximum at pH 7·2. Sensitivity in these circumstances is such that 0·4 p.p.m. of Hg^{2+} can be visually detected. According to Feigl's definition,[2] the dilution limit and the limit of identification are, respectively, $1{\cdot}1 \times 10^8$ and 0·4 μg.

Experimental

Effect of pH on the light absorption of the complex—

A suitable zincon reagent solution was prepared by dissolving 0·13 g of zincon in 2 ml of *N* sodium hydroxide and diluting to 100 ml with water. Aqueous solutions containing a given constant amount of Hg^{2+} (as mercuric chloride) in volumes of approximately 40 ml were adjusted to various pH values by dropwise addition of 0·1 *N* hydrochloric acid or 0·1 *N* sodium hydroxide. After the addition of 2 ml of zincon reagent solution to each, the solutions were diluted accurately to 50 ml with water. One aliquot was then taken for a final pH check and one aliquot was used for the spectrophotometric reading. Absorption curves were taken over the wavelength range of 400 mμ to 700 mμ at various pH values from 3·4 to 10·3. Absorption maxima occurred at about 600 mμ, but tended to be displaced to shorter wavelengths below pH 5·0. Table I summarises the more useful information gained from this series of experiments.

Table I

Extinction values at various wavelengths and pH values for a Hg^{2+} concentration of 4 p.p.m.

	Extinction at a wavelength of							
pH	580 mμ	590 mμ	595 mμ	600 mμ	605 mμ	610 mμ	615 mμ	620 mμ
3·4	0·325	0·317	0·310	0·30	0·285	0·26	0·23	0·20
5·4	0·146	0·16	0·16	0·16	0·155	0·155	0·155	0·155
7·2	0·31	0·34	0·346	0·35	0·35	0·35	0·346	0·345
8·0	0·285	0·305	0·312	0·312	0·312	0·312	0·311	0·310
9·1	0·147	0·160	0·160	0·161	0·157	0·157	0·155	0·154

Choice of buffer solutions—

The buffer solution chosen for establishing a pH of 7·2 was prepared from 13·616 g of potassium dihydrogen phosphate and 69·8 ml of *N* sodium hydroxide diluted to 2 litres with water free from carbon dioxide. Other buffer solutions were examined, because of expected difficulties due to precipitation of insoluble phosphates. These included a barbitone - hydrochloric acid buffer, a borax - boric acid - sodium chloride buffer (Palitzsch's)

and Sorensen's borax - hydrochloric acid buffer, but they either partly or completely inhibited the colour reaction. Later, however, it was found that a Clark and Lubs pH 8·2 buffer (boric acid, potassium chloride and sodium hydroxide) could be used with only slight loss of sensitivity and this modification is mentioned under "Method."

COMPOSITION OF THE COLOURED COMPLEX—

The ratio of metal to reagent in the resulting complex was determined by the molar-ratio method[3] and also by the slope-ratio method.[4] At pH values of 7·2 and 9·0, the latter established with a Clark and Lubs buffer solution, the ratio was found to be: Hg^{2+} to zincon = 1 to 2.

TABLE II

INTERFERENCE OF FOREIGN IONS

In all tests 4 p.p.m. of Hg^{2+} were added initially

Foreign ion	Molar ratio of foreign ion to Hg^{2+}	Hg^{2+} found, p.p.m.
Mg^{2+}	10	3·4
Mg^{2+}	1	3·8
Ca^{2+}	100	2·4*
Ca^{2+}	1	4·0
Sr^{2+}	1	4·0
Ba^{2+}	1	4·0
Sb^{3+}	1	0·09†
Sb^{5+}	100	2·2
Sb^{5+}	10	3·9
As^{5+}	1000	4·0
As^{3+}	10	2·4
As^{3+}	1	4·0
Sn^{2+}	1	0·08‡
Sn^{4+}	100	1·7
Sn^{4+}	1	4·0
Fe^{2+}	1	1·6
Fe^{3+}	1	3·8
Fe^{3+}	100	2·7
Pb^{2+}	1	4·1
Pb^{2+}	100	1·1*
NH_4^+	10	3·9
NH_4^+	1000	1·7
Al^{3+}	1	3·5
Al^{3+}	100	1·4*
Be^{2+}	100	4·0
Bi^{3+}	1	1·1§
Cd^{2+}	10	3·9
Co^{2+}	10	5·1
Ni^{2+}	10	3·1
Ni^{2+}	1	4·2
Cr^{3+}	10	4·0
Cr^{3+}	100	3·9
Mn^{2+}	1	3·6
Mn^{2+}	10	1·0‖
CN^-	1	0 ¶
SO_4^{2-}	100	4·0
NO_3^-	100	4·0
Acetate	10	4·0
Acetate	100	3·8

* = solution filtered.
† = solution deep orange colour.
‡ = solution orange red colour.
§ = solution orange colour.
‖ = solution green colour.
¶ = solution deep orange colour.

INTERFERENCE FROM FOREIGN IONS—

Interferences under conditions otherwise suitable for determining mercury were examined as follows.

Suitable volumes of "foreign ion" solutions (at concentrations of 0·1 *M*, 0·01 *M* or 0·001 *M*) were added to 50-ml calibrated flasks containing 1 ml of 0·001 *M* Hg^{2+} and 30 ml

of pH 7·2 buffer solution. After the addition of 3 ml of zincon reagent solution, each was diluted to the mark with pH 7·2 buffer solution and the optical densities were measured at a wavelength of 600 mμ with a Beckman DU spectrophotometer, a 1-cm cell and a reference blank consisting of 3 ml of the zincon reagent solution made to up to 50 ml, pH 7·2 buffer solution being used. This is desirable, because the reagent itself absorbs appreciably at this wavelength. Any precipitates formed were filtered off before measurement of the optical densities.

Table II shows the results of these experiments. It will be noted that neither chloride nor sulphate show any interference, and, at least in this respect, zincon offers an advantage over *p*-dimethylaminobenzylidenerhodanine.[5] Zinc and copper were not included in the experiments, since they are known to produce intense colours with the reagent under these conditions.

Method

Reagent—

Zincon reagent solution—Dissolve 0·13 g of zincon in 2 ml of *N* sodium hydroxide and dilute to 100 ml with water.

Procedure—

Transfer a suitable aliquot of solution, containing 100 to 400 μg of Hg^{2+} and adjusted to pH 7·2 by addition of 0·1 *N* hydrochloric acid or 0·1 *N* sodium hydroxide to a 50-ml calibrated flask. Add 3 ml of zincon reagent solution and dilute to the mark with pH 7·2 buffer. Various amounts of buffer solution from 10 to 40 ml give no significant variation to the optical densities.

Measure the optical density at 600 mμ in a 1-cm cell, using 3 ml of zincon reagent solution made up to 50 ml with pH 7·2 buffer solution as reagent blank and read the mercury concentration from a calibration graph (previously prepared with use of known amounts of mercury).

Calibration graphs were found to be linear over the range 100 to 400 μg of Hg^{2+} and day-to-day reproducibility was found to be satisfactory without resorting to rigid temperature controls. In the presence of ions yielding insoluble phosphates a Clark and Lubs pH 8·2 buffer solution may be substituted in the procedure with only slight loss of sensitivity.

The behaviour of certain foreign ions in this modified method is shown in Table III. These experiments were otherwise conducted precisely as those referred to in Table II.

Table III

Interference of foreign ions

In all tests 4 p.p.m. of Hg^{2+} were added initially

Foreign ions	Molar ratio of foreign ion to Hg^{2+}	Hg^{2+} found, p.p.m.
Ca^{2+}	100	3·9
Ca^{2+}	1000	3·1
Sr^{2+}	100	3·9
Sr^{2+}	1000	3·1
Ba^{2+}	100	3·6
Mn^{2+}	1	1·6*
Ni^{2+}	100	4·7
Co^{2+}	1	4·1
Co^{2+}	10	6·5
Cd^{2+}	100	5·3
Bi^{3+}	1	> 15†

* = solution green colour.
† = solution deep purple.

Detection and visual estimation of minimal amounts of mercury

Adjust the pH of the test solution to pH 7·2 and dilute to 50 ml in a Nessler tube with pH 7·2 buffer solution. Add 0·25 ml of diluted zincon reagent solution (the reagent solution diluted tenfold with water) from a microburette and compare in a matched Nessler tube with a control containing identical amounts of buffer solution and reagent only.

The presence of minimal amounts of mercury is indicated by the test solution showing a slight "greying" by comparison with the control. As the concentration of mercury increases, this "greying" becomes recognisable as a gradual increase in blue colour.

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Organo-tin - Dithizone Complexes

The Colorimetric Determination of Diethyltin and Triethyltin Compounds

By W. N. Aldridge and Jill E. Cremer

It has been demonstrated that some organo-tin compounds react with dithizone. The absorption maximum for the diethyltin - dithizone complex, after being shaken with 10 per cent. trichloroacetic acid, is at 510 mμ, whereas triethyltin does not react under these conditions. In the presence of borate buffer of pH 8·4, diethyltin and triethyltin compounds produce with dithizone absorption maxima at 485 and 440 mμ, respectively. At 510 mμ the triethyltin - dithizone complex and dithizone have the same absorption. Based upon these findings and their distribution between chloroform and aqueous media, a method is described for the separation and determination of diethyltin and triethyltin compounds. Interference from other metals is avoided by the use of ethylenediaminetetra-acetic acid.

Recently interest has been shown in the biological activity of organo-tin compounds,[1,2] after reports of their potential use as fungicides,[3] as clearing agents in plastics and for the treatment of boils in France.[4] A method was required for the determination of the dialkyltin and trialkyltin compounds and for estimating mixtures of these compounds.

Although diphenylthiocarbazone (dithizone) is a well known reagent for detecting small amounts of metals, little use has been made of its reactivity with organo-metallic compounds. Miller, Polley and Gould[5] and later Miller and Polley[6] reported a method for determining mixtures of ethylmercuric and phenylmercuric compounds by using dithizone and stated that several other organo-mercuric compounds reacted with this reagent. Webb, Bhatia, Corwin and Sharp[7] also published analytical data on the reaction products of dithizone and organo-mercury compounds. In this paper it is shown that dithizone reacts with organo-tin compounds, and a method for the qualitative and quantitative differentiation of mixtures of triethyltin and diethyltin compounds is described.

Experimental

All optical measurements were made with a Unicam SP600 spectrophotometer.

A preliminary investigation showed that, when a solution of diethyltin dichloride was shaken with a chloroform solution of dithizone in the presence of aqueous 10 per cent. trichloroacetic acid, a red colour was produced. Tetraethyltin and triethyltin sulphate did not react under these conditions. On the other hand, when shaken with borate buffer of pH 8·4, triethyltin sulphate gave a yellow colour and diethyltin dichloride an orange colour. Under these conditions neither tetraethyltin, monoethyltin nor stannic chloride produced any change of colour.

PURITY OF DIETHYLTIN AND TRIETHYLTIN COMPOUNDS—

Triethyltin sulphate was prepared from triethyltin hydroxide supplied by The Tin Research Institute. Triethyltin hydroxide (26·2 g; 0·118 mole) was dissolved in 130 ml of acetone, the solution was filtered and 12·0 ml (0·06 mole) of 5 *M* sulphuric acid were added with vigorous stirring. A further 130 ml of acetone were added, the mixture was cooled and the solid was filtered off. After re-suspension and washing in acetone, the solid was filtered off, and dried in air and finally *in vacuo*. The yield was 23·5 g of triethyltin sulphate; polarographic analysis after wet-ashing showed that it contained 47·5 per cent. of tin (theory 46·8 per cent.). As stated above, diethyltin dichloride forms a complex with dithizone when shaken with 10 per cent. trichloroacetic acid (see Fig. 1), whereas triethyltin sulphate does not react. By means of a calibration curve prepared under these conditions from our specimen of diethyltin dichloride, it can be shown that the maximum possible content of diethyltin sulphate in our triethyltin sulphate is 0·2 per cent.

Diethyltin dichloride was used as supplied by Dr. van der Kerk (Amsterdam). Analysis gave 49·8 per cent. of tin (theory 47·8 per cent.) and 28·3 per cent. of chloride (theory 28·6 per cent.). It has been found that triethyltin sulphate distributes between *N* potassium hydroxide and chloroform in favour of the chloroform (see Table I) at a mean distribution coefficient of chloroform/*N* potassium hydroxide = 7, whereas most of the diethyltin dichloride remains in the alkali. If all the material remaining in chloroform is triethyltin, the maximum amount of triethyltin chloride in this specimen of diethyltin dichloride would be 0·5 to 0·8 per cent. In fact, visually it was apparent that the colour produced was like that of diethyltin, so that most of the 0·5 to 0·8 per cent. is undoubtedly also diethyltin.

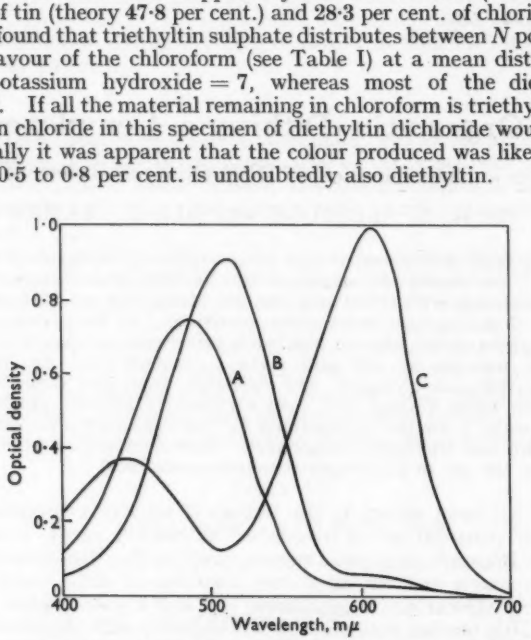

Fig. 1. Absorption spectra of diethyltin - dithizone complexes: curve A, 392 μg of diethyltin dichloride in 3 ml of borate - EDTA buffer solution (pH 8·4) shaken with 6 ml of 0·00067 per cent. w/v dithizone in chloroform; curve B, 1190 μg of diethyltin dichloride in 3 ml of 10 per cent. w/v trichloroacetic acid (pH 0·8) shaken with 6 ml of 0·00067 per cent. w/v dithizone in chloroform; curve C, 6 ml of 0·00067 per cent. w/v dithizone in chloroform shaken either with borate - EDTA buffer solution or trichloroacetic acid. Identical spectra were obtained. Readings were made in 1-cm cells against chloroform, a Unicam SP600 spectrophotometer being used

ABSORPTION SPECTRA—

The results in Figs. 1 and 2 are for solutions prepared by mixing dithizone in chloroform with increasing amounts of the organo-tin compound until no further increase in the absorption at the maximum was obtained. Fig. 1 shows that the absorption maximum for the diethyltin - dithizone complex is at 510 mμ, and at 485 mμ after the complex has been shaken with 10 per cent. trichloroacetic acid and borate buffer of pH 8·4, respectively. Fig. 2 shows

that the absorption maximum of the triethyltin - dithizone complex, after being shaken with borate buffer of pH 8·4, is at 440 mμ. No complex is formed if triethyltin is shaken with 10 per cent. trichloroacetic acid. Measurements with the triethyltin - dithizone complex must be carried out in subdued light, since upon exposure to bright light there is a rapid change in the absorption spectrum. This change is consistent with the conversion of the triethyltin - dithizone complex to the diethyltin - dithizone complex. Further experiments upon this change are being carried out.

The sensitivity of the reaction is shown in the calibration curves (compare with Table V) and the evidence of purity makes it certain that the changes in absorption spectra shown are due to diethyltin and triethyltin and not to any impurity in the preparations.

In Fig. 1 it is clear that the extinction of the diethyltin - dithizone complex produced at the pH of 10 per cent. trichloroacetic acid is higher than that produced from borate buffer of pH 8·4. Extraction from 10 per cent. trichloroacetic acid is a more specific procedure, since triethyltin does not react under these conditions. However, considering the practical problem of determining small amounts of diethyltin, the sensitivity of the reaction under these conditions is lower. In Table II are given the differences in optical densities of dithizone and dithizone shaken with a given amount of diethyltin in either 10 per cent. trichloroacetic acid or borate buffer. Since the difference in optical density of dithizone and diethyltin - dithizone complex at the pH of 10 per cent. trichloroacetic acid at 510 mμ is large (see Fig. 1), the low reading in Table II must be due to poor extraction from the aqueous phase. This is confirmed by the demonstration that if the diethyltin is shaken into the dithizone in chloroform from borate buffer and the chloroform layer is then made acid with 1 drop of 10 per cent. ethanolic trichloroacetic acid, a much higher reading than that for direct extraction from trichloroacetic acid is obtained. In practice, however, we have found that the addition of ethanolic trichloroacetic acid is a procedure giving erratic results and it is not suitable for routine use.

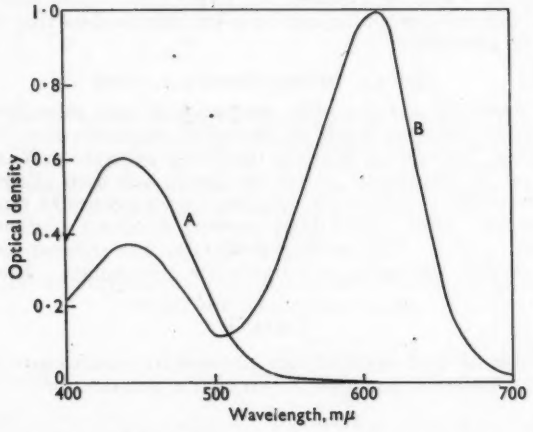

Fig. 2. Absorption spectrum of triethyltin - dithizone complex: curve A, 390 μg of triethyltin sulphate in 3 ml of borate - EDTA buffer solution (pH 8·5) shaken with 6 ml of 0·00067 per cent. w/v dithizone in chloroform; curve B, 6 ml of 0·00067 per cent. w/v dithizone in chloroform shaken with 3 ml of borate - EDTA buffer solution.

Fig. 2 shows that at 510 mμ, the minimum for dithizone, the triethyltin - dithizone complex has the same absorption. Reading the absorption at 510 mμ will therefore be a specific measure of diethyltin in a mixture of these two compounds. It is essential however to remove interference from other reacting metals. The extinction of the triethyltin - dithizone complex is low and if readings are made at 610 mμ some method of separation of the two compounds is necessary.

INTERFERENCE FROM OTHER METALS—

Dithizone is a non-specific reagent and reacts with a large number of metals. However, ethylenediaminetetra-acetic acid (EDTA) has very little effect upon the reaction of diethyltin and triethyltin with dithizone at pH 8·4 (Table III). This has proved extremely useful, for it eliminates interference from many metallic ions. Tests have shown that with use of borate buffer containing 0·25 per cent. of EDTA no interference is encountered in the presence of 500 μg of cupric, zinc, ferrous, ferric, lead, manganous, cobalt and stannic ions. Mercuric ions interfere under these conditions.

SEPARATION OF DIETHYLTIN AND TRIETHYLTIN COMPOUNDS—

As stated earlier, if diethyltin and triethyltin in chloroform are shaken with potassium hydroxide, the diethyltin partitions into the alkali while most of the triethyltin passes into the chloroform. Separation is also obtained between chloroform and borate buffer with or without EDTA. Some results shown in Table IV indicate that the distribution coefficient for borate to chloroform is approximately 50 for diethyltin and 0·13 for triethyltin. The separation of diethyltin from triethyltin is almost complete, and triethyltin may be measured after addition of dithizone to the chloroform by the reduction in absorption at 610 mμ. The absence of diethyltin may be demonstrated by the lack of absorption at 510 mμ. The concentration of diethyltin in the aqueous phase may be determined by re-shaking with dithizone in chloroform and measuring the absorption at 510 mμ. The small amount of triethyltin present in the aqueous phase does not interfere at this wavelength.

OTHER ORGANO-TIN COMPOUNDS—

Several other dialkyltin and trialkyltin compounds have been briefly examined. It has been found that several of the trialkyltin compounds were impure and contained appreciable quantities of the dialkyltin compound. It is clear from the results obtained so far that diethyltin, dibutyltin and dioctyltin all produce absorption spectra with maxima at 485 mμ from borate buffer of pH 8·4 and at 510 mμ from 10 per cent. trichloroacetic acid. Sufficient work has been done with tri-*n*-propyltin and tri-*n*-butyltin to show that they produce similar absorption spectra to triethyltin.

RESULTS OF EXPERIMENTAL WORK

Distribution of triethyltin and diethyltin compounds between chloroform and N *potassium hydroxide*—A chloroform solution (5 ml) of diethyltin dichloride was shaken with 3 ml of *N* potassium hydroxide. A solution (3 ml) of triethyltin sulphate in *N* potassium hydroxide was shaken with 5 ml of chloroform. After the layers had been allowed to separate, the aqueous layer was removed by suction, the tube was rinsed down with water and the aqueous layer was again removed. Then 1 ml of 0·004 per cent. dithizone solution and 3 ml of borate buffer of pH 8·4 were added. The reading at 610 mμ was referred to calibration curves prepared from the original diethyltin and triethyltin compounds. The results are shown in Table I.

TABLE I

DISTRIBUTION OF TRIETHYLTIN AND DIETHYLTIN COMPOUNDS BETWEEN CHLOROFORM AND *N* POTASSIUM HYDROXIDE

Tin compound	Total amount of organo-tin compound, μg	Amount in chloroform (5 ml) after extraction, μg	Amount in 3 ml of *N* potassium hydroxide after extraction (by difference), μg
Diethyltin dichloride..	300	2·2	298
	600	2·7	597
	900	5·2	895
	1200	8·1	1192
Triethyltin sulphate ..	6	5·0	1·0
	18	16·5	1·5
	30	28·3	1·7

Sensitivity of the reaction of diethyltin with dithizone under various conditions—A solution containing 12 μg of diethyltin dichloride (1 ml) was added to 3 ml of borate buffer of pH 8·4 or 3 ml of 10 per cent. trichloroacetic acid of pH 0·8. After the solution had been mixed

with 6 ml of 0·00067 per cent. w/v dithizone in chloroform, the optical density was read in 1-cm cuvettes against the dithizone control. The results are shown in Table II.

TABLE II

SENSITIVITY OF THE REACTION OF DIETHYLTIN WITH DITHIZONE UNDER VARIOUS CONDITIONS

Conditions	Optical density at 495 mμ	510 mμ	610 mμ
Extracted from 10 per cent. trichloroacetic acid ..	0·06	0·07	0·088
Extracted from borate buffer of pH 8·4	0·31	0·25	0·54
Extracted from borate buffer of pH 8·4; chloroform layer was separated and 1 drop of 10 per cent. ethanolic trichloroacetic acid was added	0·21	0·24	0·28

Effect of EDTA on the reaction of dithizone with diethyltin and triethyltin—A solution of the organo-tin compound in water (1 ml) was added to 3 ml of borate buffer with or without 0·25 per cent. w/v of EDTA (disodium salt). The solution was mixed thoroughly for 1 minute with 6 ml of 0·00067 per cent. w/v dithizone in chloroform, and then the optical density was read against the dithizone control. The results are shown in Table III.

TABLE III

EFFECT OF EDTA ON THE REACTION OF DITHIZONE WITH DIETHYLTIN AND TRIETHYLTIN

Tin compound	Amount present, μg	Optical density for borate buffer at 510 mμ	610 mμ	Optical density for borate - EDTA buffer at 510 mμ	610 mμ
Triethyltin	5·9	—	0·16	—	0·17
	11·8	—	0·31	—	0·34
Diethyltin	2·6	0·11	—	0·09	—
	5·2	0·20	—	0·17	—

Distribution of diethyltin and triethyltin between borate buffer of pH 8·4 and chloroform—A solution of the organo-tin compound (1 ml) was added to 3 ml of borate buffer of pH 8·4, and shaken with 5 ml of chloroform. After removal of the aqueous layer, the tube was rinsed with water, which was then removed, and the organo-tin content of the chloroform layer was determined. The results are shown in Table IV.

TABLE IV

DISTRIBUTION OF DIETHYLTIN AND TRIETHYLTIN BETWEEN BORATE BUFFER OF pH 8·4 AND CHLOROFORM

Tin compound	Amount taken, μg	Amount in chloroform layer, μg	Distribution coefficient for borate to chloroform
Diethyltin	300	7·8	48
	900	20	55
Triethyltin	12	10·8	0·14
	18	16·5	0·12
	24	21·8	0·13
	30	26·8	0·12

METHOD

REAGENT—

Dithizone, 0·004 per cent. w/v in chloroform—This solution should be stored in the dark at + 5° C.

Borate - EDTA buffer of pH 8·40—Dissolve 19 g of sodium borate, $Na_2B_4O_9.10H_2O$, 12 g of boric acid and 2·5 g of disodium ethylenediaminetetra-acetate in distilled water and dilute to 1 litre.

Organo-tin compounds—Stock solutions (50 mg per 100 ml) of triethyltin sulphate and diethyltin dichloride were prepared in distilled water. These solutions contain 405 μg of triethyltin per ml and 357 μg of diethyltin per ml, respectively.

Purification of reagents—Dithizone (obtained from The British Drug Houses Ltd.) was purified as described by Sandell,[8] except that after acidification the solid was filtered on a glass sinter, washed free from acid and dried *in vacuo* over sulphuric acid. This preparation has a molar extinction coefficient in chloroform at 610 mμ of $36{\cdot}4 \times 10^3$. Chloroform, B.P., was used as supplied by Hopkin & Williams Ltd., but chloroform residues were recovered by distillation after treatment with sulphuric acid and lime by the method of Sandell.[8]

TABLE V

CALIBRATION RESULTS FOR THE SEPARATION AND DETERMINATION OF TRIETHYLTIN AND DIETHYLTIN

Results for triethyltin—						
Triethyltin present, μg	5·9	11·8	17·7	23·6	29·5	
Optical density at 610 mμ	0·115	0·235	0·360	0·460	0·555	
Optical density at 510 mμ	0·01	0·01	0·01	0·004	0	
Results for diethyltin—						
Diethyltin present, μg	5·1	10·3	15·4	20·6	25·7	
Optical density at 510 mμ	0·115	0·22	0·305	0·35	0·38	

TABLE VI

DETERMINATION OF TRIETHYLTIN AND DIETHYLTIN IN MIXTURES

Triethyltin taken, μg	29·5	23·6	17·7	11·8	5·9	
Diethyltin taken, μg	0	5·1	10·3	15·4	20·6	
Triethyltin found, μg	28·7	23·2	17·5	12·0	6·2	
Diethyltin found, μg	0·5	5·1	9·2	14·7	20·0	

PROCEDURE—

To 1 ml of aqueous solution containing up to 24 μg of organo-tin compounds add 3 ml of borate - EDTA buffer and 5 ml of chloroform. Mix well by moving a mushroom-ended glass rod up and down for 1 minute. Diethyltin remains in the aqueous phase. Allow the layers to separate, take 3 ml of this phase, add 5 ml of chloroform and 1 ml of dithizone reagent and again mix for 1 minute. Remove the aqueous layer by suction. Read the difference in absorption between a control and the unknown at 510 mμ. The reading so obtained is referred to a calibration curve prepared in an identical manner (see Table V) from standard solutions of diethyltin.

The chloroform phase from the original distribution contains 90 to 91 per cent. of the triethyltin. Remove the remaining borate - EDTA buffer and take 4 ml of the chloroform layer. Add 1 ml of chloroform, 1 ml of dithizone reagent and 3 ml of borate - EDTA buffer. In subdued light again mix thoroughly with the mushroom-ended glass rod for 1 minute, and remove the aqueous layer by suction. The difference in absorption between the control and unknown at 610 mμ is referred to a calibration curve prepared in an identical manner (see Table V) from standard solutions of triethyltin. It may be readily shown that at 510 mμ there is no diethyltin present. If the triethyltin - dithizone complex in chloroform is exposed to bright light, absorption at 510 mμ becomes apparent.

Results for the calibration curves are shown in Table V. The relation between triethyltin and optical density at 610 mμ is linear up to 30 μg, whereas that for diethyltin is not, and in practice under these conditions not more than 20 μg of diethyltin should be taken. The range of the determination of diethyltin may be increased by using more dithizone.

RESULTS

In Table VI are shown the results obtained in analyses of mixtures of triethyltin and diethyltin. Good recoveries are obtained. If only diethyltin or triethyltin are present singly, then the initial distribution between chloroform and borate buffer may be omitted.

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TOXICOLOGY RESEARCH UNIT
MEDICAL RESEARCH COUNCIL LABORATORIES
CARSHALTON, SURREY

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Polarographic Determination of Tin in Foods

BY J. MARKLAND AND F. C. SHENTON

A polarographic method has been developed for the routine determination of tin in canned foods. Recovery experiments indicated that with 1-mg quantities of tin the accuracy was about ± 3 per cent. Interference due to residual nitric acid has been avoided by heating the acid digest with ammonium oxalate.

MANY methods have been proposed for the determination of tin in foods, but most of them have disadvantages for routine work.

A gravimetric method[1] needs large samples and involves tedious separations.

Volumetric methods[2,3] depending on the reducing action of stannous tin are subject to interference by oxygen from the air or dissolved in reagents. Owe[3] reduced tin in an inert atmosphere with aluminium and subsequently oxidised it with 0·02 *N* iodine. He allowed for the oxygen dissolved in the iodine solution by using an empirical factor. Vogel[4] has recommended the use of air-free iodine and suggested that if this was not done the errors might amount to 10 per cent. when 0·01 *N* iodine was used.

Colorimetric methods depending on the reduction of complex molybdates by stannous tin[5,6] need elimination of oxygen from the atmosphere or reagents[7] in the same way as volumetric methods. Dithiol methods have been improved by the use of dispersing agents,[8,9] but separation of copper is needed and the dithiol reagent is not very stable.

Polarography has advantages in speed and specificity. Godar and Alexander[10] determined tin in foods by polarography after acid digestion, co-precipitation on aluminium hydroxide and solution of the precipitate in hydrochloric acid. These authors had some failures when applying the polarographic method to acid digests without the co-precipitation stage. These failures were shown to be due to interference with the tin wave by residual nitric acid from the acid digestion.

Preliminary experiments on the polarographic determination of tin in solutions of foods obtained by nitric - sulphuric acid digestions confirmed the findings of Godar and Alexander.[10] In some instances we obtained indistinct waves that overlapped the tin wave. This effect could be reproduced by incomplete removal of the nitric acid. Owe[3] removed the last traces of nitric acid by heating with ammonium oxalate before volumetric determination of tin, and we have applied this treatment to the acid digests before polarography. By this means the nitric acid interference has been eliminated.

Godar and Alexander[10] used an H-type polarographic cell and suggested that in the simple cell the anode pool reduced some stannic tin. This may be true, but we have used a simple cell and obtained results for standard tin solutions that gave a straight-line plot of diffusion current against concentration. The straight line was reproducible.

METHOD

APPARATUS—

Electrochemical Laboratories Manual Polarograph Model B Mark II. Capillary diameter, 0·06 mm. Drop time, 2·9 seconds. Rate of flow of mercury (m), 2·59 mg per second. Height

of mercury column (h), 30 cm. With a 5-g sample and the instrument on half sensitivity, up to 400 p.p.m. of tin can be conveniently determined.

REAGENTS—

Sulphuric acid, concentrated.
Nitric acid, concentrated.
Ammonium oxalate, saturated solution.
Supporting electrolyte—Dissolve 53·5 g of ammonium chloride in distilled water, add 89 ml of concentrated hydrochloric acid and dilute to 1 litre with distilled water.
Gelatin—A 1 per cent. w/v solution in distilled water.

PREPARATION OF SAMPLE—

Thoroughly mix the whole contents of the can, preferably in a macerator. If a macerator is used, some of the more solid packs must be diluted with an equal weight of water.

PROCEDURE—

To about 5 g of prepared sample in a 300-ml Kjeldahl flask add 10 ml of concentrated nitric acid and boil to low bulk. Add 4 ml of concentrated sulphuric acid and heat to incipient charring. To the boiling solution add further concentrated nitric acid dropwise from a separating funnel until digestion is complete; about 5 ml of nitric acid are usually sufficient. Continue heating until fumes of sulphuric acid are observed. Cool, add 10 ml of saturated ammonium oxalate solution, and heat to fuming. Repeat the ammonium oxalate treatment. Transfer the solution to a 50-ml calibrated flask, rinsing the flask with supporting electrolyte, add 1 ml of gelatin solution, and adjust to volume at 20° C with the supporting electrolyte. Under our laboratory conditions this temperature is maintained during polarography without further adjustment. Transfer some of the solution to the polarograph cell, de-aerate with nitrogen and record the polarogram of the solution between − 0·3 and − 0·8 volt, using full compensation current to give a better shaped curve. Under these conditions tin has a half-wave potential of about − 0·5 volt against the mercury pool anode and gives a calibration line—

$$\text{Tin, mg} = 1{\cdot}35 \times i_d, \quad i_d \text{ being measured in } \mu\text{A.}$$

Lead gives a wave indistinguishable from tin, but zinc, copper, manganese and iron do not interfere. This interference of lead is of no importance in the routine examination of foods, since the amount of lead that is significant for rejection of a sample is so much smaller than that of tin. If sufficient lead is present in a sample to interfere significantly with the tin determination, the sample would be rejected because of lead contamination. With modern cans such a degree of lead contamination is most unlikely. No lead determinations were made on the samples reported in Tables II and III.

RESULTS

Experiments with known amounts of tin either alone or with other metals are summarised in Table I. The recovery of 106 per cent. in experiment 6 is explained by the presence of 0·10 mg of lead.

TABLE I

POLAROGRAPHIC DETERMINATION OF TIN

Experiment No.	Tin taken, mg	Other metals taken	Tin found, mg	Recovery, %
1	0·50	—	0·49	98
2	1·00	—	0·99	99
3	1·00	—	1·00	100
4	0·25	0·025 mg of Pb; 0·25 mg of Cu; 0·50 mg of Zn; 0·50 mg of Fe	0·24	96
5	0·50	0·025 mg of Pb; 0·25 mg of Cu; 0·50 mg of Zn; 0·50 mg of Fe	0·49	98
6	0·50	0·10 mg of Pb; 0·30 mg of Cu; 1·00 mg of Zn; 1·00 mg of Fe; 0·40 mg of Mn	0·53	106

The method was used for the analysis of samples to which known amounts of tin had been added. The original tin content of these samples was determined by the same method. The results are given in Table II.

TABLE II

RECOVERY OF TIN FROM FOODS

Experiment No.	Sample	Tin present, mg	Tin added, mg	Tin found, mg	Recovery of of added tin, %
7	Meat soup + 0·5 mg of Pb; 0·15 mg of Cu; 0·50 mg of Zn; 0·50 mg of Fe	0·21	1·25	1·51	104
8	Stewed steak	0·02	0·10	0·125	105
9	Herrings	0·075	0·50	0·555	96
10	Herrings	0·05	0·75	0·79	99
11	Herrings	0·15	1·25	1·38	98
12	Tomatoes	0·30	1·00	1·30	100
13	Creamed rice	0·05	1·00	1·02	97
14	Peaches	0·49	0·50	1·00	102
15	Grapefruit	0·15	0·50	0·65	100

The method was further checked by comparison with Owe's volumetric method.[3] The results are given in Table III.

TABLE III

COMPARISON OF RESULTS

Experiment No.	Sample	Tin found by Owe's method, p.p.m.	Tin found by polarographic method, p.p.m.
16	Prunes	505	505
17	Tomato puree	258	254
18	Milk + 100 p.p.m. of tin	102	99

Preliminary trials on the analysis of organo-tin compounds by this method gave 33·9 per cent. of tin in hexaphenylditin (theory 33·9 per cent.) and 27·0 per cent. of tin in triphenyltin bromide (theory 27·6 per cent.).

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The Polarographic Determination of Tin in Hydrogen Peroxide after use of a Cation-exchange Resin

By G. F. REYNOLDS

The development of a polarographic method for the determination of tin, added as a stabiliser to concentrated hydrogen peroxide, is described. The method involves the use of a cation-exchange resin column, the preparation and packing of which are given in detail. Results are presented and certain aspects are discussed.

Tin, usually in the form of sodium stannate, is commonly employed as a stabiliser for concentrated hydrogen peroxide, and numerous methods have been evolved for its determination. The most satisfactory of these employ a polarographic finish, but none appears to have been published.

All these polarographic methods, of which details are known, involve use of a decomposition procedure to destroy the peroxide. This either entails boiling in acid solution in the presence of a platinum catalyst, or boiling in alkaline solution. In the former method total destruction of the peroxide takes a considerable time and tin is often lost in the process. The latter method is somewhat more satisfactory, but in neither is there means of positive assurance that decomposition is complete.

Work in this Department on the determination of copper in concentrated hydrogen peroxide[1] had shown that this element could be removed from the bulk of the hydrogen peroxide by means of a cation-exchange resin, thus reducing the time needed for total decomposition. Further experiment had indicated that this procedure might also be applied to the separation of tin.[2] The work described in this paper was therefore undertaken to develop a method for the determination of tin in concentrated peroxide by means of an ion-exchange separation and a polarographic finish.

All polarography was carried out at 25° C on a Cambridge direct-writing polarograph and all potentials are quoted in the European sign convention. On this convention the potential of the saturated-calomel electrode has been taken as + 0·246 volt against the normal hydrogen electrode.

Experimental

Preliminary experiments—

On the basis of previous work Zeo-Karb 225 cation-exchange resin (50 to 100 mesh) was chosen for this determination. A glass tube 35 cm long and of external diameter 1 cm, having a funnel and burette tap sealed to the upper and lower ends, respectively, was employed to contain the column. This tube had a plug of glass-wool inserted just above the tap to act as a "seat" for the resin.

The resin was conditioned as follows—

> The smallest particles of the material were first removed by a process of elutriation and a 25-cm column of packed resin was then prepared by pouring an aqueous slurry into the tube, the particles being allowed to settle and the supernatant water run off. This was successively eluted with 400 ml of 2 *N* hydrochloric acid, 50 ml of water, 400 ml of 10 per cent. ammonium chloride solution and finally 300 ml of water. The resin was then transferred to a 250-ml conical flask and boiled for 10 minutes with 100 ml of 100-volume hydrogen peroxide. No decomposition was observed.

The conditioned Zeo-Karb 225 was washed three times by decantation and packed into the column as before until a length of 15 to 20 cm was obtained. Finally the column was eluted with 25 ml of 5 *M* hydrochloric acid and washed with 100 ml of water. The liquid flow through this column was satisfactorily rapid and even; 25 ml of 0·05 *M* sulphuric acid passed through in about 3 minutes.

Experiments with 50-ml volumes of solution, each containing 10 μg of tin per ml and concentrations varying from 1·0 *M* to 0·01 *M*, showed that the recovery of tin was markedly affected by the initial acid concentration. Recoveries ranged from about 7 per cent. for

solutions 1·0 *M* in sulphuric acid to a fairly steady value of 60 to 70 per cent. for solutions containing 0·025 *M* sulphuric acid or less. These results were obtained polarographically, after elution of the tin from the column with 10 ml of 5 *M* hydrochloric acid into a 25-ml calibrated flask containing 0·25 ml of 0·1 per cent. proteose peptone as maximum suppressor. The solution in the flask was then made up to volume with washings from the column. Tin waves of good shape were obtained with half-wave potentials of 0·475 volt against the mercury pool.

In view of these results the initial concentration of sulphuric acid was fixed at 0·02 *M*, since loss of tin owing to hydrolysis had been encountered at concentrations less than this value. Further experiments were then carried out with the object of increasing the efficiency of the extraction and elution procedures. Modifications made as a result of these experiments were—

(*a*) To minimise hydrolysis of the tin while in the column, with consequent difficulty in elution, the wash with water before elution was replaced by the passage of 20 ml of 0·02 *M* sulphuric acid through the column.

(*b*) The volume of hydrochloric acid used for elution was found to be barely adequate. It was also shown that recoveries were increased if the eluting acid was re-cycled through the resin. In all further work, therefore, elution was carried out with 15 ml of 5 *M* hydrochloric acid, which was passed three times through the column.

Recoveries of tin under these conditions were better than 95 per cent.

A series of solutions of volume 50 ml, 0·02 *M* with respect to sulphuric acid and containing 0 to 25 μg of tin per ml, was next prepared and treated as follows—

The solution was passed through the column, which was then washed with 20 ml of 0·02 *M* sulphuric acid. The tin was eluted with 15 ml of 5 *M* hydrochloric acid, which was passed three times through the column and finally collected in a 25-ml calibrated flask containing 0·25 ml of 0·1 per cent. proteose peptone as maximum suppressor. The column was then washed with water, which was used to dilute the solution in the flask to the mark.

The steps recorded for these solutions were of good shape and were of half-wave potential −0·465 volt against the mercury pool. The heights of the steps were linear with concentration within the range 0·25 to 15 μg per ml. At higher concentrations the linear relationship began to fail (see "Discussion of results," p. 49).

APPLICATION TO SAMPLES OF HYDROGEN PEROXIDE—

Although the bulk of the hydrogen peroxide was removed by passage of the solution through the column, it appeared certain that traces would remain on the resin, even after washing. As this would give rise to an extended wave,[3] which might interfere with the tin step, it was necessary to include a procedure for its destruction. Sulphur dioxide was found to be a very satisfactory reagent for this purpose.

Four 20-ml aliquots of a sample of 90 per cent. hydrogen peroxide were placed in 50-ml calibrated flasks and aliquots of a standard tin solution in 0·5 *M* sulphuric acid containing 0, 50, 100 and 200 μg of tin, respectively, were added. Sufficient sulphuric acid was then added to each flask to give a final concentration of 0·02 *M* and the contents were then made up to volume with water. The following procedure was carried out on each solution—

The solution was passed through a column prepared as already described and was then discarded. The 50-ml calibrated flask was rinsed with 20 ml of 0·02 *M* sulphuric acid, which was also passed through the column and then discarded. The tin was eluted with 15 ml of 5 *M* hydrochloric acid, which was collected in a 100-ml beaker. This acid was returned to the top of the column twice and allowed to pass through again into the beaker. The column was finally washed with 25 ml of water, which was also collected in the beaker. Sulphur dioxide gas from a syphon was passed through the solution for 5 minutes, and the solution was then boiled vigorously in the presence of an "anti-bump" rod until the volume was reduced to about 10 ml. Care was taken to ensure that the odour of sulphur dioxide was absent at this stage. If still present, 25 ml of water were added and the evaporation was repeated. The contents of the beaker were finally transferred to a 25-ml calibrated flask containing 0·25 ml of 0·1 per cent. proteose peptone and made up to volume with water. An aliquot of this solution was withdrawn, deoxygenated and a polarogram was recorded.

The steps obtained from the solutions were well shaped and had half-wave potentials that varied between −0·46 and −0·475 volt against the mercury pool. The recoveries are given in Table I and a typical step is shown in Fig. 1.

TABLE I

RECOVERY OF TIN IN HYDROGEN PEROXIDE

Tin added, μg	Equivalent concentration of tin in hydrogen peroxide, p.p.m.	Step height (sensitivity 1/10), mm	Tin found, p.p.m.
0	0·0	16·5	(13·75)
50	2·5	19·0	15·83
100	5·0	22·0	18·75
200	10·0	28·5	23·75

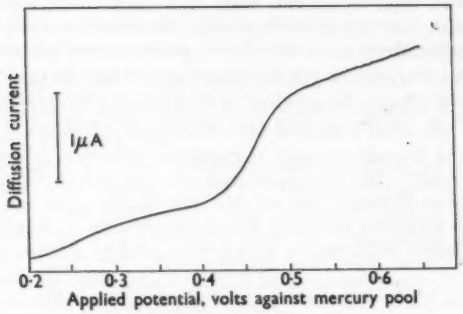

Fig. 1. Polarogram for a solution containing 20 μg of tin per ml in approximately 3 *M* hydrochloric acid, after ion-exchange separation from hydrogen peroxide (equivalent to 16 p.p.m.)

These results were considered to be satisfactory and the procedure was therefore incorporated into the proposed method.

METHOD

Place two 20-ml aliquots of the hydrogen peroxide sample in 50-ml calibrated flasks and add to one of them 10 ml of 0·1 *M* sulphuric acid. To the other flask add 1·0 ml of a standard solution containing 200 μg of tin (Note 1) and 5 ml of 0·1 *M* sulphuric acid. Make up to volume with water and proceed with each solution as follows.

Pass the solution through an ion-exchange column, 0·75 cm in diameter and containing a 15-cm length of packed Zeo-Karb 225 prepared and conditioned as previously described. Wash out the calibrated flask with 20 ml of 0·02 *M* sulphuric acid and pass the washings through the column. Discard the solution and washings.

Pour 15 ml of 5 *M* hydrochloric acid through the column and collect the eluate in a 100-ml beaker. Re-cycle the eluate twice more and finally wash the column with 20 ml of water, collecting the washings in the beaker. Pass sulphur dioxide through the solution for 5 minutes and then boil in the presence of an "anti-bump" rod until the volume is reduced to about 10 ml (Note 2). Allow to cool. Transfer the solution to a 25-ml calibrated flask, add 0·25 ml of 0·1 per cent. proteose peptone and make up to volume with washings from the beaker.

Place an aliquot of the solution in a polarographic cell, deoxygenate and record a polarogram at 25° C, using the mercury pool as anode. Measure the step that occurs at about −0·46 volt.

Use the heights of the step obtained with the two solutions to calculate the tin content of the hydrogen peroxide.

NOTES—

1. *Standard tin solution*—Weigh out 0·50 g of pure tin into a 250-ml beaker. Add 25 ml of water, followed by 25 ml of 18 *M* sulphuric acid. Boil gently until all the tin has dissolved. Carefully add a further 50 ml of 18 *M* sulphuric acid, a little at a time, cooling after each addition. Dilute to about 200 ml with water and, while still warm, transfer to a 250-ml calibrated flask. Make up to volume with washings from the beaker. Cool and re-make to volume, if necessary.

By pipette put 10·0 ml of the prepared solution into a 100-ml calibrated flask and make up to volume with water, when—

$$1 \text{ ml} \equiv 200 \ \mu\text{g of tin.}$$

These solutions have poor keeping qualities and must be renewed monthly or whenever they become cloudy.

2. The odour of sulphur dioxide should be absent at this stage. If this is not so, a further 20 ml of water should be added and the evaporation repeated.

TESTS OF THE METHOD

In order to test the accuracy and reproducibility of the method described above, determinations were made on a number of samples of concentrated hydrogen peroxides, both in the absence and presence of added tin. The results are given in Table II.

TABLE II

ACCURACY OF THE METHOD

Tin added, p.p.m.	Tin found in—				
	sample 1, p.p.m.	sample 2, p.p.m.	sample 3, p.p.m.	sample 4, p.p.m.	sample 5, p.p.m.
0	14·5	11·7	17·8	15·5	8·2
5	19·0	17·0	21·6	20·0	14·0
10	24·5	21·5	27·8	24·8	18·5

DISCUSSION OF RESULTS

The proposed method of removing the tin from hydrogen peroxide renders a "fuming down" procedure unnecessary. Such a procedure was previously necessary to destroy organic materials added occasionally, as stabilisers, in addition to tin. These now pass out of the column with the bulk of the peroxide. Determinations on a number of peroxide samples known to contain organic stabilisers, such as 8-hydroxyquinoline and benzoic acid, have shown that these cause no interference.

The tedious preparation of the resin has been found to be necessary for the removal of impurities. The main impurity appears to be iron. It is also necessary to convert the resin to the hydrogen form and to ensure that no attack by hydrogen peroxide will take place during the determination. The only effect of the hydrogen peroxide on the resin was found to be physical; that of the release of oxygen as it passes through the column. This causes bubbles to form in the packed resin, which seriously interfere with liquid flow. They must be removed by shaking after each determination, if results are to be reproducible. The correctly prepared column keeps indefinitely.

In the initial experiments the 10 ml of 5 *M* hydrochloric acid used for elution gave a final concentration of 2 *M* hydrochloric acid, which was known to be satisfactory for the polarography of tin. The half-wave potential found (− 0·475 volt) is in good agreement with that quoted in the literature.[4] The change in half-wave potential observed when the volume of eluting acid was increased to 15 ml (final concentration 3 *M*) is entirely due to the change in the anode-pool potential from + 0·02 volt to + 0·01 volt against the saturated-calomel electrode. Similarly the variation in half-wave potential observed during determinations is evidently due to losses of hydrochloric acid during evaporation. These changes, however, are insufficient to cause significant variation in the viscosity of the solution, so that measurable changes in the step-height - concentration relationship will not occur.

The destruction of traces of residual peroxide by sulphur dioxide proceeds in accordance with the equation—

$$H_2O_2 + SO_2 = H_2SO_4.$$

This is a convenient method because the reaction is vigorous and complete and because the odour of excess of sulphur dioxide indicates completion. The excess of sulphur dioxide is completely removed by vigorous boiling, which is a necessity, otherwise this compound would itself give rise to an interfering reduction step.

The results obtained in the preliminary experiments showed that with more than 15 μg of tin per ml of the final solution there is a "fall off" in the linear relationship between step height and concentration, and this becomes serious at 25 μg per ml. This, however, corresponds to 36·25 p.p.m. of tin in the original sample, which is more than twice the amount usually added as a stabiliser. Linearity may be re-established by lengthening the resin column together with other minor modifications.

I thank J. Walkden and K. Cottell for assistance in the early stages of the work.

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Developments in the Micro Vacuum Fusion Method

With particular reference to the Determination of Oxygen, Nitrogen and Hydrogen in Beryllium, Titanium, Zirconium, Thorium and Uranium

By E. BOOTH, F. J. BRYANT and A. PARKER

The application of the micro vacuum fusion technique to the determination of oxygen, nitrogen and hydrogen in boron, beryllium, copper, chromium, iron, silicon, tantalum, thorium, titanium, uranium and zirconium has been examined. With sample weights in the range 10 to 100 mg, oxygen and hydrogen have been determined with a precision and accuracy comparable with that usually obtained on the decigram to gram scale.

Evidence is presented to support the view that the vacuum fusion determination of nitrogen in thorium, titanium, zirconium and uranium is often unreliable.

In recent years many papers have been published on the subject of the vacuum fusion determination of gases in metals. The development of the technique owes much to the pioneering work of Sloman and his co-workers at the National Physical Laboratory,[1,2,3] who successfully demonstrated the use of this method in the analysis of iron and steel, some 20 years ago. More recently, these workers have extended their enquiries to metals such as titanium, zirconium, molybdenum, vanadium and uranium.[4] A comprehensive bibliography of the subject is given by Yeaton,[5] and there have been important contributions by Mallett *et al.*[6,7] and by Gregory *et al.*[8,9]

We have extended the investigations started by Gregory and have applied the method to a variety of metals, including several of current metallurgical importance.

Description of the Apparatus

The apparatus is based on a design described by Gregory, Mapper and Woodward.[8]

Furnace assembly—

The graphite crucible, heat shield and silica furnace assembly are similar to those used by Gregory, Mapper and Woodward. A Radyne C150 induction heater rated at 15 kilowatts (450 kilocycles) is used to heat the crucible. With a maximum indicated output of 8½ to 9 kilowatts a crucible temperature of 2400° C is obtainable. The use of such a large generator is necessitated by the small area (1/8th) of work coil occupied by the crucible, and the omission

of the graphite powder insulation commonly employed. However, the additional cost of the large generator is more than offset by the ease and rapidity of assembly, dismantling and outgassing, and this design has proved entirely satisfactory in practice.

The sample loading mechanism has been simplified. Twelve samples can be accommodated in the "tree" and can be released by raising retaining plungers magnetically. The samples are guided into the crucible by means of a silica funnel, which is lowered by magnetic neutralisation of its counterweight. The material for the metal bath is stored in the four side-arms on the furnace assembly and can be dropped when required by the raising of the retaining plungers.

Temperatures are measured by means of a disappearing-filament-type optical pyrometer, the crucible being observed through a quartz optical flat fitted into a side-arm of the furnace tube. Corrections to the observed temperatures due to absorption by the prism and window were determined from measurements on a standard lamp certified by the National Physical Laboratory. These corrections ranged from less than 10° C at 1600° C and + 40° C at 1800° C to + 100° C at 2100° C. All temperatures quoted in this paper have been corrected.

GAS TRANSFER AND ANALYSIS SYSTEM—

This is similar in basic design to that described by Gregory, Mapper and Woodward. Additions include a Pirani gauge head, a bulb containing Hopcalite and a palladium tube that can be heated to 350° to 400° C by means of a moveable furnace. These items were found to be necessary for a modified method of gas analysis, which is described in detail in a later section.

It is most important that the gases evolved from the sample should be removed from the furnace tube as rapidly as possible, to minimise losses due to chemisorption, or other reactions with the hot graphite or evaporated metal films. This requirement has been achieved by connecting a high-speed two-stage mercury-diffusion pump to the furnace tube assembly by wide-bore tubing (35 mm in diameter). A further requirement of this transfer pump is that it shall have a reasonably high critical backing pressure, since the pressure in the collecting volume may occasionally build up to 1 to 2 mm. The pump used has a critical backing pressure of 4 mm and a speed of 6 litres per second, at 10^{-4} mm of mercury. The trap between the pump and the furnace tube has proved to be unnecessary and has been omitted in a second apparatus. We have also found that it is unnecessary to place gold foil between the pump and furnace tube to prevent the forward diffusion of mercury.

The evolved gases are confined between the backing side of the transfer pump and mercury cut-offs of the type used by Gregory. Six auxiliary collecting bulbs were installed for experimental purposes, but are not required for routine analysis. Since it was the intention to employ a low-pressure method of gas analysis *in situ*, no provision was made for collecting the gases at atmospheric pressure. This feature could, if required, be incorporated into the apparatus without difficulty.

GENERAL VACUUM SYSTEM—

The system is evacuated by a two-stage mercury-diffusion pump backed by a mechanical rotary pump. The type of the latter is immaterial, since there are no large volumes of gas to be pumped. Provision is made for all parts of the apparatus to be evacuated simultaneously to avoid mercury "fly-over." A second mechanical pump provides ancillary services, such as the lowering of mercury in the cut-offs and McLeod gauge.

PRESSURE MEASUREMENTS AND GAS ANALYSIS—

Pressure measurements are made with a double scale McLeod gauge (10^{-5} to 10^{-1} and 10^{-1} to 1 mm of mercury) or alternatively with a Pirani gauge. The latter is invaluable for checking the completeness of gas evolution and for following the course of the gas analysis. In order to relate pressure measurements to gas content of the sample, the volume in which the gases are collected must be accurately known. This calibration is accomplished by measuring the pressure, P_1, and volume, V_1, of a suitable amount of dry oxygen confined in precision-bore tubing in the gas burette, this gas being then pumped into the collecting volume, V_2, and its pressure, P_2, measured. Then, since $V_2 = P_1V_1/P_2$, the required volume can be calculated. By making some twenty such measurements, the error in calibration was calculated to be not greater than 1 per cent. based on a 95 per cent. confidence limit, the value of V_2 being 800 ml.

The low-pressure methods of gas analysis now in common use have much to recommend them as regards speed and accuracy and we have used them exclusively. The gas mixture to be analysed consists of carbon monoxide, hydrogen and nitrogen, with occasionally 1 or 2 per cent. of carbon dioxide. In preliminary work, a combustion and fractional-freezing method of analysis was used. In this, a known volume of oxygen is added to the mixture and the carbon monoxide and hydrogen are oxidised to carbon dioxide and water at a platinum filament heated to 500° C. The products of combustion are frozen out in a side-arm cooled in liquid nitrogen. If the side-arm is then raised to — 78° C by immersion in a slurry of acetone and solid carbon dioxide, the carbon dioxide previously frozen out will sublime, and its pressure can then be measured. By simple calculation the amounts of carbon monoxide, hydrogen and nitrogen in the original gas can be determined.

This method was not entirely satisfactory, in that oxygen was adsorbed by the platinum filament, and it was necessary to determine the unadsorbed oxygen by the addition of hydrogen and further combustion. Also, the nitrogen result, being calculated by difference, was unduly influenced by small measuring errors. Further, if the volume of gas collected was larger than expected, the analysis became relatively time-consuming, since for a minor constituent, *e.g.*, nitrogen, it was not feasible to take an aliquot of the mixture for analysis.

The method subsequently adopted was that devised by Davis and Gray.[10] In this, carbon monoxide is oxidised to carbon dioxide by means of Hopcalite, an activated manganese dioxide (manufactured by Siebe Gorman Ltd.). This reagent accomplishes the oxidation rapidly (2 to 3 minutes) at room temperature without the addition of oxygen. As in the combustion method, the resulting carbon dioxide is frozen out in a liquid-nitrogen trap. Hydrogen is determined by measuring the drop in pressure when the gas mixture is brought into contact with a palladium tube heated at 350° to 400° C. The residual gas is assumed to be nitrogen. The complete analysis takes about 10 minutes and the method is, in our opinion, unequalled for this type of analysis.

General operation of the method

The fundamentals of the vacuum-fusion technique, together with a consideration of the thermodynamics involved, have been discussed by several authors, notably by Sloman, Harvey and Kubaschewski[4] and by Brewer.[11] It is generally agreed that a mobile liquid-metal bath containing an adequate amount of dissolved carbon is a pre-requisite for the efficient operation of the method. The desirable characteristics of the metal employed as the "bath" are that—

(*a*) it should remain liquid and not form a solid carbide at the operating temperature,
(*b*) it should be more noble towards oxygen and carbon than the metal being analysed,
(*c*) it should be of low volatility and a poor "getter," and
(*d*) it should readily dissolve the sample metal.

From these considerations, suitable metals appear to be platinum, palladium, cobalt, iron and nickel. Of these, iron is the most commonly used, although, owing to the lower solubility of carbon in it and its lower vapour pressure, platinum is much superior. Since upwards of 80 per cent. of the platinum is recoverable, the cost is not prohibitive and we have used it almost exclusively and very successfully.

Before attempting a detailed discussion of the application of the method to various metals, an outline of the procedure in the general case will be given, and what follows is a typical example, assuming the sample to be in the form of a rod or an ingot.

Samples (usually in duplicate) are prepared by cutting approximately to size with a hacksaw and abrading the surface with a file. After degreasing, they are weighed and stored in a desiccator. The samples and a suitable weight of platinum in the form of short lengths of 14 s.w.g. wire are loaded into the "tree" and side-arm, respectively. The furnace tube, fitted with a new crucible and "dolly" and the "tree" are placed in position, all joints being made with Picien wax. When the apparatus has been evacuated to about 10^{-3} mm, the induction heater is switched on and the crucible is heated at 800° to 1000° C for a few minutes to remove the loosely held moisture and air. Thereafter the temperature is raised over a period of about 10 minutes to 2150° to 2200° C and maintained at this level until the blank rate is acceptable. This usually takes about 1½ to 2 hours.

Higher outgassing temperatures (up to 2400° C) can be obtained, but above 2200° C the rate of evaporation of graphite becomes excessive, and in one instance, while outgassing at

2350° to 2400° C, the furnace tube cracked, owing, it is believed, to a conducting film of condensed graphite being formed. When the outgassing of the graphite is completed, the crucible is allowed to cool to below 1000° C and the platinum to form the "bath" is added. The temperature is raised to 1860° ± 20° C over a period of 2 to 3 minutes, and the platinum is outgassed for 15 to 20 minutes at this temperature. The blank gas is now collected for 10 minutes and analysed, the composition being typically 30 to 40 per cent. of carbon monoxide, 60 to 50 per cent. of hydrogen and 10 per cent. of nitrogen. The average value of the blank rate at 1860° C is equivalent to 0·066 ml per hour at S.T.P., *i.e.*, about 0·02 ml of carbon monoxide per hour. This is usually negligible, for example, on a metal having a 0·1 per cent. oxygen content the blank would be 1 per cent. of the carbon monoxide collected from a 40-mg sample.

The precise technique used for the addition of the sample and collection of gas varies somewhat with the type of material and the analysis required. For tantalum, thorium, titanium and zirconium the crucible can be maintained at the operating temperature throughout the run, since no trouble due to metal evaporation is encountered. With beryllium, however, it appears to be essential to introduce the sample into the crucible at a temperature below 1200° C, and to switch the heater off between sample additions. With uranium also, if more than 6 to 8 samples are to be analysed it is preferable to switch off between samples.

The evolution of gas from the sample is usually complete in less than 1 minute, but a collection period of 2 to 3 minutes is given to allow a wide safety margin. After the collected gases have been isolated from the furnace tube by raising a mercury cut-off the analysis is carried out, residual gas is pumped away, and the next sample is added. It is a noticeable feature of vacuum fusion work that the optimum conditions for the analysis of a particular metal are by no means agreed upon by the various workers in this field. There have even been cases in which experiments repeated by other workers under what appear to be identical conditions have given unsatisfactory results.[7] A constantly occurring difficulty in this work is the scarcity of samples of known or agreed gas content. Sloman, Harvey and Kubaschewski[4] used the oxide of the metal to be analysed to establish reduction temperatures, but in our apparatus this would involve the weighing and handling of microgram quantities, which, although not impossible, is somewhat impracticable.

Consequently, operating conditions have usually been determined from the results of a series of experiments at various temperatures, on a sample of metal. In most cases the operating temperature is not very critical, and we have found the rate of gas evolution to be a sound guide. Provided that it is rapid (not greater than 1 minute), no increase in the amount evolved or change in composition has been observed at higher temperatures.

Determinations in metals

Titanium—

From the results of exploratory experiments, an operating temperature of 1880° ± 20° C with a 3 to 5-g platinum bath appeared to be satisfactory.

Analyses were then carried out on a portion of a titanium ingot that had already been examined at the National Physical Laboratory; this was supplied by courtesy of Mr. H. A. Sloman. This material had been vacuum melted a number of times and could reasonably be expected to be homogeneous. The results are shown in Table I.

Table I

Oxygen determination in titanium

Sample weights, 10 to 30 mg; temperature, 1880° ± 20°C;
bath contained 5 g of platinum; gas-collection time, 2 minutes

N.P.L. value, %: 0·16
Oxygen found, %: 0·15(8), 0·15(9), 0·15(8), 0·15(4), 0·12(7), 0·16(0), 0·14(5), 0·15(8), 0·15(0), 0·15(6), 0·12(4), 0·14(4)
Mean = 0·14(9); standard deviation = 0·012

These results, which were obtained consecutively in one run, are in good agreement with the N.P.L. figure, and show that satisfactory precision is obtainable with sample weights of the order of one-tenth of those commonly used. Another titanium ingot also analysed by the N.P.L. and by another worker in this field gave the results shown in Table II.

TABLE II

OXYGEN DETERMINATION IN TITANIUM

Sample weights, 7 to 12 mg; temperature, 1880° ± 20°C;
bath contained 5 g of platinum; gas-collection time, 2 minutes

N.P.L. value, %: 0·21
Oxygen found, %: 0·21(4), 0·24(8), 0·22(9), 0·19(3), 0·22(0), 0·22(8), 0·18(0), 0·22(0), 0·21(5), 0·20(7), 0·21(6)
Mean = 0·21(5); standard deviation = 0·018
Oxygen found by other workers, %: 0·22(0), 0·22(0), 0·22(0), 0·24(0), 0·21(0)
Mean = 0·22(2); standard deviation = 0·011

In some samples examined, the spread of results was considerably greater than those shown in Tables I and II. However, the results on the macro scale were on these samples likewise less precise, the spread, as would be expected, being somewhat smaller.

An example of this is given in Table III. This sample of a titanium ingot was also supplied through the courtesy of Mr. H. A. Sloman.

TABLE III

OXYGEN DETERMINATION IN TITANIUM

Sample weights, 20 to 60 mg; temperature, 1880° ± 20° C;
bath contained 5 g of platinum; gas-collection time, 2 to 3 minutes

N.P.L. values, %: 0·05(4), 0·08(7), 0·06(0), 0·05(0)
Mean = 0·06(3)
Oxygen found, %: 0·04(8), 0·06(5), 0·05(6), 0·04(7), 0·05(8), 0·05(3), 0·05(4), 0·05(5), 0·05(6), 0·05(0), 0·11(4), 0·07(5), 0·06(5), 0·08(0), 0·05(5), 0·06(6), 0·05(9), 0·06(0), 0·06(0), 0·06(6), 0·05(5), 0·06(1)
Mean = 0·06(2); standard deviation = 0·010

The determination of hydrogen in titanium presents no difficulties, the gas being evolved rapidly at temperatures above 1200° C.

The determination of nitrogen is not straightforward and will be discussed later.

ZIRCONIUM—

Initial experiments were carried out with "hard-fired" zirconia. From this very refractory material quantitative recovery of oxygen was obtained with a 3-g platinum bath at temperatures of 1900° to 1950° C. Since such materials are usually much less reactive than the oxide present in a metal, the figures quoted indicate the likely upper temperature limits required. In fact, if conditions are made favourable for carbide formation, calculations of the free-energy changes likely to occur indicate that reduction would occur at much lower temperatures, provided, of course, the approach to equilibrium conditions is rapid.

A series of experiments was then carried out on a sample of a zirconium ingot. Operating temperatures covered the range 1700° to 1950° C, and the results were in good agreement throughout, there being no trends in observed oxygen content. The evolution of gas was rapid and apparently quantitative in all tests. The standard temperature for subsequent analysis of zirconium was fixed at 1860° ± 20° C, this approximating to the melting point of the metal and being sufficiently above the melting point of platinum to give a thoroughly fluid bath.

At this stage of the investigation, the combustion method of gas analysis was replaced by the Hopcalite method, and the opportunity was taken to compare the results by both methods of analysis. Results from their application to a sample of a zirconium ingot are shown in Table IV.

Whilst the duplicate figures in Table V were obtained from adjacent portions of sample, it has been established that a similar precision is obtained on ingot samples when the material is systematically sampled both along the length and across the surface of discs cut from it. Samples of zirconium sponge have also been examined for oxygen, nitrogen and hydrogen, but sampling difficulties are such as to render the results of doubtful value. In certain cases these can be partly overcome by compacting several hundred grams of material and drilling samples at several places.

TABLE IV

OXYGEN DETERMINATION IN ZIRCONIUM

Sample weights, 30 to 60 mg; temperature, 1860° ± 20° C;
bath contained 3 g of platinum

Oxygen found by Hopcalite method, %: 0·10(7), 0·10(3), 0·09(5), 0·09(8), 0·09(9), 0·10(5), 0·09(8), 0·09(8), 0·09(5), 0·09(0), 0·09(9), 0·09(5)
Mean = 0·09(8); standard deviation = 0·006
Oxygen found by combustion method, %: 0·10(2), 0·09(8), 0·10(3), 0·09(2), 0·09(5), 0·09(6), 0·09(2), 0·09(5), 0·10(2)
Mean = 0·09(8); standard deviation = 0·004

Several hundred analyses have been carried out on zirconium and zirconium alloys, and the results shown below in Table V are typical of those obtained on re-melted ingots.

TABLE V

OXYGEN DETERMINATION IN ZIRCONIUM-ALLOY INGOTS

Sample weights, 20 to 60 mg; temperature, 1860° ± 20° C;
bath contained 3 g of platinum

Sample	Oxygen found, %	Sample	Oxygen found, %
1	0·11(0), 0·11(0)	6	0·10(8), 0·10(1)
2	0·09(1), 0·08(4)	7	0·13(6), 0·15(8)
3	0·12(4), 0·11(7)	8	0·10(8), 0·09(7)
4	0·13(4), 0·12(4)	9	0·10(2), 0·10(5)
5	0·07(7), 0·07(7)	10	0·10(5), 0·10(6)

As with titanium, there is no difficulty in the determination of hydrogen, the gas being rapidly and quantitatively evolved at temperatures above 1200° C. The determination of nitrogen is unsatisfactory and is discussed later.

THORIUM—

Although the stability of thorium oxide necessitates a high temperature for its reduction, the low vapour pressure of the metal at such temperatures, together with the stability of the carbide, permit the determination of oxygen to be carried out fairly easily. The results of initial experiments on various samples showed that gas evolution was rapid and complete at temperatures of 1850° to 1950° C. The precision was similar (coefficient of variation 5 to 10 per cent.) to that of the determinations on zirconium.

To assess the accuracy of the determination of oxygen, two samples from thorium bars previously analysed by Mr. Sloman at the N.P.L. were examined, with the results shown in Table VI.

TABLE VI

OXYGEN DETERMINATION IN THORIUM

Sample weights, 10 to 60 mg; temperature, 1900° ± 20° C;
bath contained 3 g of platinum

Sample No. 1—
N.P.L. value, %: 0·33
Oxygen found, %: 0·36, 0·33, 0·35, 0·34
Sample No. 2—
N.P.L. value, %: 0·14
Oxygen found, %: 0·14, 0·13, 0·13

As with zirconium, metal in the form of a rod or bar showed no marked heterogeneity with respect to oxygen, and results for thorium metal rod are shown in Table VII.

TABLE VII

OXYGEN DETERMINATION IN THORIUM ROD

Sample weight, 10 to 15 mg; temperature, 1900° ± 20° C; bath contained 3 g of platinum

Sample No. 1—
Oxygen found, %: 0·41, 0·41, 0·40, 0·40, 0·40, 0·40, 0·40, 0·40, 0·39, 0·38
Sample No. 2—
Oxygen found, %: 0·38, 0·35, 0·33, 0·39, 0·39, 0·36, 0·33, 0·34, 0·38, 0·34

On the other hand, thorium metal powders showed distinct heterogeneity with respect to oxygen content. Useful information can, however, be obtained from examination of these materials, as is shown by the results, in Table VIII, from a series of experiments in which known amounts of oxide were incorporated into a sample of thorium powder and the recoveries were checked.

TABLE VIII

OXYGEN DETERMINATION IN THORIUM POWDER

Sample weights, 15 to 25 mg; temperature, 1900° ± 20° C; bath contained 3 g of platinum

Original powder—
Oxygen found, %: 0·53, 0·48, 0·44, 0·32, 0·45
Mean = 0·44
Original powder + 0·12% *of oxygen*—
Oxygen found, %: 0·53, 0·43, 0·72, 0·57, 0·60
Mean = 0·57
Original powder + 0·30% *of oxygen*—
Oxygen found, %: 0·72, 0·54, 0·81, 0·65, 0·60
Mean = 0·66
Original powder + 0·60% *of oxygen*—
Oxygen found, %: 1·09, 1·05, 0·79, 1·10, 0·94, 0·84
Mean = 0·97

Although powders are somewhat inconvenient to handle in our apparatus, they can be dealt with by enclosure in thin-walled platinum tubing. The somewhat high blank obtained from this tubing can be tolerated, since the oxygen content of the samples is usually several tenths per cent. As with titanium and zirconium, the determination of hydrogen in thorium has been straightforward, whereas that of nitrogen has for the most part been unsuccessful.

URANIUM—

The vacuum fusion technique has been successfully applied to the analysis of uranium by a number of workers. Using a platinum bath at 1820° ± 20° C, we have found gas evolution to be rapid and quantitative, as judged by agreement obtained on samples analysed elsewhere. The relatively high volatility of uranium in comparison with zirconium or thorium would appear to be responsible for the occasionally observed decrease in apparent oxygen content when the uranium - platinum ratio in the crucible exceeded about 30 per cent. w/w. On samples of uranium rod, the precision obtained was similar to that observed for zirconium or thorium, as shown by the results in Table IX.

TABLE IX

OXYGEN DETERMINATION IN URANIUM ROD

Sample weights, 6 to 200 mg; temperature, 1820° ± 20° C; bath contained 3 g of platinum

Sample No. 1—
Oxygen found, %: 0·011, 0·011, 0·014, 0·013, 0·011, 0·018
Sample No. 2—
Oxygen found, %: 0·018, 0·015, 0·013, 0·019, 0·014

Again, the determination of hydrogen is relatively simple, and that of nitrogen not entirely satisfactory.

TANTALUM—

From a consideration of the free energy of formation of Ta_2O_5, and the great stability of the carbide, it was thought that satisfactory results could be expected under conditions shown to be suitable for zirconium. This appeared to be so for the small number of samples examined, and gas evolution was rapid at 1860° C. Circumstances, however, did not permit a thorough investigation of operating conditions.

Some typical results are given in Table X.

TABLE X

OXYGEN DETERMINATION IN TANTALUM

Sample weights, 25 to 75 mg; temperature, 1860° ± 20° C; bath contained 3 g of platinum

Sample No.	Oxygen found, %	Sample No.	Oxygen found, %
1	0·028, 0·022	6	0·034, 0·031
2	0·013, 0·010	7	0·032, 0·037
3	0·027, 0·032	8	0·040, 0·041
4	0·025, 0·032	9	0·18, 0·15, 0·17
5	0·028, 0·029		

BERYLLIUM—

Although Gregory and Mapper[9] developed what appeared to be a satisfactory technique for the analysis of beryllium, we have not so far been able to duplicate their findings except in a limited number of instances. Examination of a sample of beryllium powder analysed by these authors and also by other workers[13] gave variable results, the mean of which was in fair agreement with the published figure. When, however, the technique was applied to samples of solid metal, results were very erratic and were not reproducible. After discussion with other workers interested in this problem, a sample of rod was produced from vacuum-melted beryllium and a series of experiments was made with this material. Despite variations in the gas-collecting time, weight of platinum bath, operating temperature, method and temperature of addition of the sample, the results remained unsatisfactory, and in particular were usually little more than 50 per cent. of a figure determined by a chemical method, which should give a reliable result at this level of oxygen content.[14] However, from the results of these experiments certain points became clear.

Firstly, the sample must be added when the crucible temperature is less than 1200° C, as addition of the samples at temperatures above the melting point of beryllium gave very low results. Secondly, the fit of the crucible lid proved to be of great importance. In one series of experiments in which the lid did not seal completely, the observed oxygen content fell from a figure of the order of 0·1 per cent. to $< 0{\cdot}001$ per cent.

At the temperature necessary for the oxide reduction the high vapour pressure (about 10 mm) of the metal causes marked evaporation with the consequent danger of chemisorption. This effect would be minimised if rapid solution of the sample and conversion to carbide could be achieved. This, however, is difficult owing to the large difference in density between beryllium and platinum. Somewhat higher and more consistent results were obtained by adding platinum and powdered graphite before the addition of each sample, but the method is still under investigation and its accuracy and reliability remain to be established.

OTHER MATERIALS—

Analyses have been successfully carried out on boron, copper, chromium, silicon and steel. For these elements, for which the required reduction temperatures are in the region of 1500° to 1600° C, an iron bath has been employed. Care must be exercised when using an iron bath to avoid the crucible lid becoming cemented to the crucible owing to slight splashing of the molten iron. The behaviour and results obtained for these metals were

in general comparable with those previously described for titanium, zirconium and so on in a platinum bath.

Determination of nitrogen

It is generally considered that the determination of nitrogen by the vacuum fusion method involves the thermal decomposition of nitrides to yield nitrogen.

From the published data, which are admittedly sometimes not very reliable, one can calculate the temperature, according to the reaction mechanism postulated, at which the equilibrium partial pressure of nitrogen should be high enough to ensure rapid decomposition of the nitride. Examples of such calculations are given in the Appendix (see p. 60) for thorium and zirconium nitrides, from the results of which it would appear that the temperatures we have employed should ensure complete and rapid removal of nitrogen.

However, in the course of preliminary work on thorium, a significant difference was discovered between the results obtained by the chemical (Kjeldahl) method and those by vacuum fusion. The results shown in Table XI were obtained on samples of thorium previously analysed at the National Physical Laboratory, and on which good agreement with the N.P.L. had been obtained for the oxygen content (see Table VI).

Table XI

Nitrogen determination in thorium

Sample No. 1—
N.P.L. value, p.p.m.: 500
Nitrogen found by vacuum fusion, p.p.m.: 120, 100, 50, 70, 80, 190, 120, 30
Nitrogen found by Kjeldahl method, p.p.m.: 220

Sample No. 2—
N.P.L. value, p.p.m.: 500
Nitrogen found by vacuum fusion, p.p.m.: 100, 60, 25, 40, 40, 75, 100
Nitrogen found by Kjeldahl method, p.p.m.: 200

Sample No. 3—
N.P.L. value, p.p.m.: 700
Nitrogen found by vacuum fusion, p.p.m.: 70, 30, 30, 30, 40
Nitrogen found by Kjeldahl method, p.p.m.: 100

These and other experiments covered considerable variations in experimental conditions, including a wide range of collection periods, temperatures and bath compositions. However, we have failed to discover conditions that would give consistent agreement with the chemical figures, nor has the discrepancy between the latter and the N.P.L. vacuum fusion figures been resolved.

Table XII

Nitrogen determination in metals

Titanium ingot A—
Nitrogen by vacuum fusion method, p.p.m.: 100, 140, 140, 140, 150, 50, 70
Nitrogen by Kjeldahl method, p.p.m.: 140, 170

Titanium ingot B—
Nitrogen by vacuum fusion method, p.p.m.: 100 to 500 (other workers found 300 to 500)
Nitrogen by Kjeldahl method, p.p.m.: 200

*Zirconium ingot**—
Nitrogen by vacuum fusion method, 240, 210, 220, 240, 140, 160, 170, 280, 140, 160, 190, 170, 190
Mean = 190; standard deviation = 38
Nitrogen by Kjeldahl method, p.p.m.: 250, 200, 260, 250, 230, 250, 230, 280, 270, 250, 250, 250, 260
Mean = 250; standard deviation = 19

Zirconium ingots—

Nitrogen by vacuum fusion method, p.p.m.:	40	130	75	40	60	15	40	150	140	70	110	140	300
Nitrogen by Kjeldahl method, p.p.m.:	100	150	130	90	160	30	80	400	330	120	150	160	310

Uranium rod—
Nitrogen by vacuum fusion method, p.p.m.: 15, 15, 30, 35, 15, 40, 50, 60, 20
Nitrogen by Kjeldahl method, p.p.m.: 45

* These figures were for adjacent portions of the sample.

There are numerous possible reasons why the vacuum fusion figures might be low—failure to decompose the nitride; adsorption of the liberated nitrogen by the crucible, furnace-tube walls and so on; errors in gas analysis.

We have failed to find any evidence of serious adsorption effects, as judged from the results of experiments in which known amounts of nitrogen were allowed to diffuse into the area around the hot crucible. Analysis of gas mixtures of known composition by two methods revealed no significant errors. Checks on thorium nitride gave 80 per cent. recovery of nitrogen. This somewhat low figure is probably due in part to the preparative difficulties and analysis of Th_3N_4, but the results show that there was no serious loss of nitrogen from failure to decompose the nitride. Similar results have been encountered in our work on titanium, zirconium and uranium; typical figures are shown in Table XII.

From these results it will be seen that most of the figures obtained by vacuum fusion are significantly lower than those obtained by the chemical method. This phenomenon has been noted by other workers,[12] but no convincing explanation appears to have been advanced. Also, from published accounts of experiments on various nitrides, when good recoveries of nitrogen were obtained, it appeared that good results from artificial standards could not necessarily be reproduced in work with actual metals. Accordingly, experiments were conducted in which known quantities of nitrogen were allowed to react with zirconium at various temperatures.

The procedure was briefly as follows—

A piece of zirconium of low nitrogen content was introduced into an outgassed graphite crucible, containing 3 g of platinum, at 1200° C. A known amount of nitrogen was allowed to react with the zirconium, the pressure in the system falling in 1 to 2 minutes to less than 5×10^{-4} mm of mercury. The crucible was heated rapidly to 1860° C and maintained at this temperature for 2 minutes. Analysis of the resulting gas mixture showed that the nitrogen was quantitatively recovered. When, however, the nitrogen and zirconium were allowed to react at temperatures of 1500° and 1600° C, recoveries of nitrogen were erratic. In one case even on raising the crucible temperature to 2000° C (after adsorption of nitrogen) the recovery of nitrogen was incomplete.

This investigation has not been carried further at present, but the results so far obtained indicate that the vacuum fusion technique, as at present operated, does not furnish very reliable figures for those elements forming very stable nitrides, particularly those of Group IVA —titanium, zirconium and thorium.

Conclusions

It is generally agreed that the vacuum fusion method lends itself to numerous variations in apparatus design and methods of gas analysis, and that many of these must remain matters of personal preference. Nevertheless, sufficient experience has been gained with our apparatus to enable us to draw the conclusions in the following sections.

Size of sample—

If the homogeneity of the sample is such that a coefficient of variation of 5 to 10 per cent. is obtainable in determinations made with 0·1 to 5-g samples, then a similar precision can be expected with samples of 10 to 20 mg.

Ease and speed of operation—

Apart from sample preparation, 12 determinations can be made in 1½ working days. This includes loading the samples into the apparatus, outgassing the graphite and the gas analysis of the 12 samples.

If a duplicate furnace tube and "tree" are available, the outgassing for the next batch of samples can be started within ½ hour of the completion of the previous analysis.

These times compare very favourably with any others of which we have cognizance and could be still further reduced by arranging for the crucible outgassing to proceed overnight.

Applicability—

We have not attempted to examine all the metals in which users of the vacuum fusion technique might be interested, but the apparatus has been used successfully for the determination of oxygen and hydrogen in boron, copper, chromium, iron, silicon, tantalum,

titanium, thorium, uranium and zirconium. There is little doubt that determinations on molybdenum, vanadium, niobium and tungsten would prove equally successful.

The range of gas contents encountered to date is approximately 0·001 to 3 per cent. for oxygen, 0·0005 to 1 per cent. for hydrogen and 0·001 to 0·1 per cent. for nitrogen. The lower limits for accurate determination will depend on the size and reproducibility of the blank, the sample weight and the effects of surface contamination.

These factors are at present under investigation, but as a guide it is expected that oxygen and hydrogen contents of 0·0005 per cent. could be determined to $\pm$ 0·0001 per cent.

The determination of oxygen in beryllium has so far proved unsuccessful and work is still in progress.

APPENDIX

Decomposition of nitrides

Although the mechanism whereby nitrides are decomposed is not known with any certainty, there appear to be three important cases to consider—

(*i*) the straightforward decomposition into metal and nitrogen,

(*ii*) decomposition into metal and nitrogen followed by solution of the metal in the liquid-metal bath, and

(*iii*) reaction with carbon, whereby the nitride is transformed to carbide and nitrogen is evolved.

These, in the case of thorium, may, following Sloman, Harvey and Kubaschewski,[4] be represented thus—

(*i*) $\langle \frac{1}{2}\ Th_3N_4 \rangle = 3/2\ \langle Th \rangle + (N_2)$,
$\Delta G = 155{,}200 - 44{\cdot}85T$ calories;

(*ii*) $\langle Th \rangle = [Th]_{Fe}$,
$\Delta G = -6000 - 7{\cdot}8T$ calories;

(*iii*) $\langle Th \rangle + 2\ \langle C \rangle = \langle ThC_2 \rangle$,
$\Delta G = -43{,}800 + 4{\cdot}0T$ calories;

where ΔG represents the algebraic increase in the free energy of the reaction and T the absolute temperature.

But $-\Delta G = \boldsymbol{R}\, T \log K$,

where K is the equilibrium constant of the reaction, in this case $\equiv p_{N_2}$,

whence $-\Delta G = 4{\cdot}57\ T \log p_{N_2}$

From (*i*) at 2173° K, $p_{N_2} = 1{\cdot}16 \times 10^{-3}$ mm of mercury,

from (*i*) and (*ii*) at 2173° K, $p_{N_2} = 3{\cdot}43$ mm of mercury,

from (*i*) and (*iii*) at 2173° K, $p_{N_2} = 234$ mm of mercury.

Owing to uncertainties in the data, these calculations can be no more than approximations. Further, in many of the experiments, platinum and not iron was used as the metal bath and no data for the free energies of solution of metals in platinum could be discovered.

Nevertheless, the fact that nitrogen removal from thorium appears to be incomplete at temperatures at which the partial pressure of nitrogen is relatively enormous seems to indicate that either a kinetic factor makes the approach to equilibrium pressures very slow, or that the equations and compounds used in the calculations are seriously in error.

For zirconium the calculations yield results that indicate a similar state of affairs, *viz.*—

(*i*) for $\langle ZrN \rangle = \langle Zr \rangle + (N_2)$,
p_{N_2} at 2130° K $= 4{\cdot}6 \times 10^{-5}$ mm of mercury;

(*ii*) for $\langle ZrN \rangle = \langle Zr \rangle + (N_2)$,
plus $\langle Zr \rangle = [Zr]_{Fe}$,
p_{N_2} at 2130° K $= 0{\cdot}8$ mm of mercury;

(*iii*) for $2\ \langle ZrN \rangle + 2\ \langle C \rangle = 2\ \langle ZrC \rangle + (N_2)$,
p_{N_2} at 2130° K $\simeq 10^7$ mm of mercury.

We acknowledge our indebtedness to Mr. A. Gunn and Mr. J. A. Piesse, whose glass-blowing and general assistance in the design and construction of the apparatus was a major factor in the success of this project.

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A Rapid Photometric Procedure for the Determination of Thiamine with 6-Aminothymol

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A simple and rapid procedure is described for the determination of thiamine in certain pharmaceutical and cereal products; it depends on the intense yellow colour produced on treatment of the vitamin with diazotised 6-aminothymol. The solubility of the colour in alkaline media, together with lower sensitivity to interfering substances and precise experimental conditions, renders this reagent more suitable for routine work than *p*-amino-acetophenone. The method is applicable to materials containing upwards of 0·1 mg thiamine per g and the maximum experimental error of a single determination is 1·5 per cent.

A SUITABLE rapid method was required for the routine analysis of cereal enrichment materials and pharmaceutical tablets containing between 0·5 and 3·5 per cent. of thiamine. The thiochrome procedure is not ideally suitable for rapid control work and it was considered therefore, that a colorimetric method of assay was more likely to meet the requirements of the laboratory dealing with large numbers of routine samples.

Numerous reagents have been proposed for the colorimetric determination of thiamine by diazotisation and coupling in an alkaline medium.[1,2,3,4,5,6] Of these, *p*-aminoacetophenone has found the most widespread application in cereal[7] and pharmaceutical work,[8,9] and a procedure based on the use of this reagent was official in the United States Pharmacopoeia XII. The latter method represented a modification of that of Auerbach,[10] in which toluene or xylene was used to extract the red dyestuff after coupling at an elevated temperature. Auerbach observed that a great variety of substances were capable of reducing the intensity of the colour produced, particularly at the higher temperatures employed to accelerate the coupling process. Elvidge[11] reported that errors as high as 13 per cent. were obtainable when the method was applied to comparatively pure solutions of thiamine, and later, Ballard and Ballard[12] showed that, to obtain reproducible results, it was necessary to work at higher levels of thiamine and to allow the colour to develop for a longer period at room temperature.

Preliminary work with *p*-aminoacetophenone confirmed the findings of other workers with regard to reproducibility and interference from natural impurities in the sample extracts. The coupling of thiamine with diazotised *p*-aminoacetophenone at higher temperatures emphasised the effects of extraneous substances, and recovery values were largely dependent on the choice of experimental conditions. On the other hand, the technique of Ballard and Ballard reduced interference at the expense of sensitivity. Various modifications of the *p*-aminoacetophenone method were briefly investigated, but in general, results were low and reproducibility was poor.

In the course of this investigation, several amines were examined from the point of view of selectivity and sensitivity to thiamine. Of the compounds tested, 6-aminothymol showed the most promise as a substitute for *p*-aminoacetophenone, and proved to be a more reliable reagent for the determination of the vitamin in the presence of sugars and soluble proteins. The photometric measurement of the yellow dyestuff produced when this reagent is diazotised and coupled with thiamine at room temperature was subsequently adopted as a suitable procedure for the rapid determination of the vitamin in enrichment materials and tablets. The method offers the advantages of simplicity and rapidity in dealing with large numbers of samples, without the need for precisely controlled experimental conditions.

Experimental

Development and stability of colour—

The colour was developed at room temperature by treating 1·0 ml of solution containing 50 μg of thiamine with 1·0 ml of freshly prepared diazo reagent. For photometric measurement this solution was diluted with water to 5·0 ml. The effects of time of standing before and after dilution are shown in Table I.

Table I

Effects of time on colour before and after dilution

Time before dilution, minutes	Optical density	Time after dilution, minutes	Optical density
½	0·37	0	0·40
1	0·385	15	0·40
2	0·40	30	0·40
5	0·40	60	0·39
10	0·40	120	0·37
30	0·375	180	0·35

Five minutes was selected as a suitable time of standing before dilution, and the values shown on the right-hand side in Table I are for the solution diluted after 5 minutes. After 30-minutes standing, the colour began to fade slowly unless extracted into an organic solvent. Extraction of the colour was incomplete when toluene alone was used; but a (9 + 1) mixture of toluene and *n*-butanol readily separated the dyestuff on shaking for 10 seconds. In this solvent the colour intensity remained constant for at least 3 hours.

To determine the effect on colour intensity of the relative concentrations of diazo reagent to thiamine, 1·0-ml quantities of the same thiamine solution were treated with graded volumes of diazotised 6-aminothymol and the optical densities determined after dilution to 5·0 ml as before. Results were as follows—

Diazo reagent, ml	0·5	1·0	1·5	2·0
Optical density	0·36	0·40	0·40	0·40

It was apparent from these results that 1·0 ml of diazo reagent was sufficient to ensure maximum colour intensity in 5 minutes when working at a level of 50 μg of thiamine per ml, this figure being chosen as a convenient working level on the basis of results obtained with *p*-aminoacetophenone.

A series of calibration curves prepared according to the method described showed that, although the curves were not strictly reproducible on different days, the relationship between optical density and thiamine concentration was linear over the range 0 to 50 μg per ml. Hence, by virtue of the simplicity of the method, it was considered more convenient and reliable to incorporate standards with each set of assays than to depend upon the use of a calibration curve.

SPECIFICITY AND INTERFERENCE—

The *p*-aminoacetophenone method is subject to interference from many substances of biological significance. The more important of these include: tyrosine, tryptophane, histidine, cysteine, adrenaline, ascorbic and uric acids, reducing sugars and certain proteins. Elimination of ascorbic and uric acids may be effected by the controlled precipitation of their lead and zinc salts, respectively, as described by Alexander and Levi,[13] and of the remaining substances, only cystein and adrenaline interfered in the proposed method. These led to yellow or brownish colours that, although not extractable into toluene - *n*-butanol, apparently inhibited formation of the thiamine colour.

In tests carried out to determine the influence of a variety of substances on the colour intensity of a solution containing 50 μg of thiamine per ml, the following were without effect at concentrations up to 100 times that of the vitamin: dextrose, lactose, maltose, sucrose, wheat flour, malt flour, casein, gelatin, peptone, urea, glycerophosphates and salts of heavy metals. Similarly, no interference was observed in the presence of the following substances at 20 times the concentration of thiamine: riboflavine, nicotinic acid, nicotinamide, pyridoxine, pantothenic acid, *p*-aminobenzoic acid, creatine, xanthine, guanine, adenine, tryptophan, tyrosine and histidine.

Degradation products of thiamine were found to yield no colour with the reagent and thiamine solutions, after autoclaving or sulphite treatment, showed comparable losses when assayed by the 6-aminothymol and fluorimetric methods.

EXTRACTION OF SAMPLES—

In the analysis of enrichment materials containing metallic iron, Rubin, De Ritter, Febbraro and Jahns[14] reported losses of thiamine when the pH of the extractant was below 5·0. Extraction between pH 5·0 and 7·0 was recommended, with use of a suitable acetate or phosphate buffer solution. In the present work, however, extraction by agitation with distilled water was found to be quite adequate and, as the results in Table II show, recoveries were unchanged by the use of a more acid medium.

TABLE II

DETERMINATION OF THIAMINE IN ENRICHMENT MATERIALS

Each material had 0·78 g of thiamine added

Extraction time, minutes	Solvent	Thiamine found, g
10	cold water	0·77, 0·79
60	cold water	0·78, 0·77
10	0·1 *N* acid	0·77, 0·77
60	0·1 *N* acid	0·78, 0·78
30	pH 6·0 buffer solution	0·77

As pointed out by Ballard and Ballard,[12] the results obtained in the assay of vitamin tablets depend to some extent on the method of extraction employed. Preliminary heating with stronger mineral acid gave slightly higher results with certain samples of tablets; but generally, cold extraction with dilute hydrochloric acid in accordance with the procedure of the British Pharmacopoeia gave perfectly satisfactory results provided that the material was sufficiently well powdered.

METHOD

REAGENTS—

Sodium nitrite solution—Prepare as required by dissolving 0·1 g of sodium nitrite in water and diluting to 100 ml.

Sodium hydroxide solution—A 20 per cent. w/v solution in water.

Solvent mixture—Mix 90 ml of redistilled toluene with 10 ml of reagent grade *n*-butanol.

Hydrochloric acid, approximately 0·1 N—Dilute 10 ml of concentrated acid to 1 litre with distilled water. Use this acid in all operations where 0·1 *N* acid is stated.

6-Aminothymol solution—Dissolve 50 mg of recrystallised 6-aminothymol hydrochloride in 50 ml of 0·1 *N* hydrochloric acid and dilute with water to 100 ml. The reagent keeps for several weeks if stored in a refrigerator.

Thiamine stock solution—Dissolve 100 mg of thiamine B.P. in sufficient 0·1 *N* hydrochloric acid to produce 100 ml solution. Preserve the solution in a refrigerator.

Thiamine working standard—Prepare as required by diluting 10 ml of thiamine stock solution to 100 ml with 0·1 *N* hydrochloric acid.

$$1{\cdot}0 \text{ ml} \equiv 100 \ \mu\text{g of thiamine.}$$

EXTRACTION OF SAMPLES—

Tablets—Thoroughly powder a weighed quantity of the tablets and proceed according to the method for Aneurine Tablets of the British Pharmacopoeia. Adjust the volume of the final extract to contain 20 to 40 μg of thiamine per ml.

Enrichment materials—Shake or stir an accurately weighed sample of about 1 g with 100 ml of water for 10 minutes. Filter rapidly, rejecting the first 10 to 20 ml of filtrate, and dilute an aliquot of the clear solution with 0·1 *N* hydrochloric acid so as to contain between 20 and 40 μg of thiamine per ml.

PROCEDURE—

Into five calibrated tubes put by pipette, respectively—

(*a*) 1·0 ml of water,
(*b*) 0·8 ml of water + 0·2 ml of thiamine working standard,
(*c*) 0·7 ml of water + 0·3 ml of thiamine working standard,
(*d*) 0·6 ml of water + 0·4 ml of thiamine working standard,
(*e*) 1·0 ml of sample extract.

Cool 5·0 ml of 6-aminothymol solution in ice - water (10° C is satisfactory if the reagent is to be used without delay), and add 2·0 ml of sodium nitrite solution. Mix and set aside for ½ to 1 minute. Add 5·0 ml of sodium hydroxide solution and dilute to 20 ml. Mix, and immediately add 1·0 ml of the diazo reagent to each of the five tubes. Allow 5 minutes for the colour to reach maximum intensity, dilute to a suitable volume, and measure the optical density of each solution in a 1-cm cell. The Spekker absorptiometer fitted with OB1 filters is a suitable instrument for this purpose.

If the sample solution is naturally coloured or turbid, proceed as described up to the point of dilution of the final solutions. Add to each tube a measured volume of mixed solvent. Shake to extract the thiamine colour into the organic layer, separate, dry the solvent with a small portion of anhydrous sodium sulphate and determine the colour intensity as before.

Calculate the thiamine concentration of the test solution, using the two nearest standard readings, *e.g.*—

$$\text{Thiamine, } \mu\text{g per ml} = 30 + 10 \times \frac{(e) - (c)}{(d) - (c)}.$$

The blank reading (*a*) serves as a check on the reagents and should not exceed 0·05. After the first set of assays with the given reagents the blank may be omitted.

RESULTS

In order to assess the accuracy and reproducibility of the 6-aminothymol method, a series of tests was carried out with use of a specially prepared sample of enrichment mix. This material contained nutrients in the normal proportions, but with the omission of thiamine. To each of ten 1-g portions of the prepared sample were added 7·50 mg of thiamine, determinations then being made according to the procedure described. The ten tests, which were carried out on different days and with use of different reagents, gave the results shown in Table III.

TABLE III

RECOVERY OF THIAMINE ADDED TO ENRICHMENT MIX

Thiamine found, mg ..	7·53	7·40	7·46	7·50	7·49	7·54	7·43	7·48	7·52	7·59
Recovery, %	100·4	98·7	99·5	100·0	99·9	100·5	99·1	99·7	100·3	101·2
Thiamine added, mg ..	7·50									
Mean recovery, mg ..	7·49									
Standard deviation ..	0·055									

Maximum error (P = 0·95): ± 1·5 per cent.

A comparison of the results obtained by the 6-aminothymol, *p*-aminoacetophenone, thiochrome and tungstosilicate procedures is presented in Table IV. The low results collected under the heading "*p*-Aminoacetophenone *A*" were obtained with the use of standards of pure thiamine. "*p*-Aminoacetophenone *B*" represents results by the same method, but with compensation for interference by addition of similar proportions of the interfering substances to the reference solutions. Fair accuracy was achieved in this way, but the limitations of the method are obvious, particularly in dealing with samples of unknown composition.

TABLE IV

COMPARISON OF RESULTS OBTAINED BY FOUR METHODS

Sample	Thiamine found with—				Thiamine found by British Pharmacopoeia method, %
	6-Aminothymol, %	*p*-Aminoacetophenone *A*, %	*p*-Aminoacetophenone *B*, %	Thiochrome, %	
Vitamin B_1	1·42	1·34	1·42	—	1·42
	1·43	1·36	1·45	—	1·43
	—	1·37	1·43	—	—
Vitamin-B_1 tablets	1·25	0·89	—	—	1·24
	1·23	0·92	—	—	1·22
Vitamin-B tablets with yeast	0·07	0·05	—	0·06	—
	0·06	0·05	—	0·06	—
Vitamin-B_1 tablets	1·56	1·20	—	1·52	1·55
	1·53	1·27	—	1·54	1·53
	1·60	—	—	—	—
Vitamin-B_1 tablets	3·36	2·16	3·20	3·28	3·27
	3·20	2·19	3·16	3·34	3·20
	3·28	—	3·17	—	—
Enrichment mix 1	0·75	0·68	—	0·74	—
	0·74	0·68	—	0·75	—
Enrichment mix 2	0·74	0·66	—	0·74	—
	0·77	0·67	—	0·76	—
Enrichment mix 3	0·74	0·68	—	0·76	—
	0·75	0·69	—	0·74	—
Enrichment mix 4	0·76	0·66	0·75	0·74	—
	0·74	0·66	0·75	0·75	—
Enrichment mix 5	0·77	0·64	0·77	0·78	—
	0·79	0·64	0·78	0·78	—
Enrichment mix 6	0·78	0·52	0·76	0·78	—
	0·78	0·52	0·76	0·79	—
Enrichment mix 7	0·74	0·70	0·72	0·74	—
	0·75	0·69	0·73	0·74	—
Enrichment mix 8	0·77	—	0·77	0·78	—
	0·78	—	0·78	0·80	—
Enrichment mix 9	0·76	—	0·74	0·78	—
	0·77	—	0·75	0·76	—
Enrichment mix 10	0·79	—	0·79	0·78	—
	0·78	—	0·79	0·78	—

CONCLUSIONS

Although the fluorimetric assay for thiamine is, in general, the method of choice, the proposed colorimetric assay offers several advantages in the routine examination of vitamin tablets or cereal enrichment materials. It is more rapid and simple than the conventional procedures and is relatively insensitive to small variations in the reagents or test solutions. Moderate amounts of protein material or carbohydrates do not interfere with colour development and, for clear and colourless sample extracts, the method may be simplified further by omission of the solvent-extraction step.

I am indebted to the Directors of Novadel Ltd. for permission to publish this paper.

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Notes

DECOMPOSITION OF OXIDE MINERALS BY FUSION WITH BORAX

RESISTANCE to decomposition is characteristic of a large number of oxide minerals, particularly those containing niobium and tantalum, and those containing calcium and titanium together. Grinding to an impalpable powder is necessary to effect decomposition by fusion with potassium pyrosulphate, or by evaporation to fumes with sulphuric acid. Even so, a few particles of ore often remain unattacked. Decomposition is even less complete if an alkaline flux is employed. For the "hydrofluoric acid method" of Lawrence Smith[1] and Wells,[2] grinding to an impalpable powder is also recommended. In my experience it is often, but not always, possible to effect complete attack of many oxide minerals by setting the finely ground mineral aside overnight with hydrofluoric acid, followed by evaporation, almost to dryness, on the water bath with two or three portions of acid.

An alternative procedure, for which grinding to an impalpable degree is not necessary, but which gives rapid and complete decomposition of oxide minerals, is fusion with borax in a platinum crucible. Magnetite, ilmenite, rutile, perovskite and minerals of the earth-acid and rare-earth groups such as columbite - tantalite, samarskite, euxenite, pyrochlore and microlite are all readily decomposed by this method. Bismutotantalite, although completely attacked, should not be fused with borax in platinum, as damage to the crucible will result. By dissolving the borax melt in dilute hydrofluoric acid, the advantages of the Lawrence Smith method are retained, and the rare earths and thorium are separated as insoluble fluorides, accompanied by the major part of the calcium of such minerals as perovskite, microlite and pyrochlore. Spectrographic analysis has shown the absence of boron, titanium, niobium and tantalum from the fluoride precipitates obtained from samples of these minerals, which are known to contain calcium, thorium and rare-earth elements.

The filtrate from the fluoride precipitation contains most of the iron originally present in the mineral and all the boron not already volatilised, together with all the titanium, niobium and tantalum. Evaporation with sulphuric acid, a necessary step in the "hydrofluoric acid method" in obtaining a pyrosulphate melt, serves in this case to remove not only all the fluorine, but also all the boron as the volatile boron trifluoride. Spectrographic analyses of pyrosulphate melts so obtained have shown that boron is quantitatively removed by a single evaporation to copious fumes of sulphuric acid. Evaporation of the fluoride solution to dryness without sulphuric acid does not completely remove all the introduced boron, even if two or three evaporations are made. That the niobium, tantalum and titanium pass quantitatively into the fluoride solution is shown by the results in Table I. In each experiment 0·1 g of lime, thoria and ceria were added to the fusion mixture. The earth acids and titania were recovered by precipitation with tannic acid after separation by the method described.

TABLE I

RECOVERY OF EARTH ACIDS AND TITANIA AFTER BORAX FUSION

Nb_2O_5 added, mg		Ta_2O_5 added, mg		TiO_2 added, mg	Recovery, mg
98·4		—		—	98·6
—		95·6		—	95·2
—		—		102·5	102·5
44·7	+	47·8	+	52·6 (=145·1)	145·3

METHOD

PROCEDURE—

Accurately weigh 0·5 g of the sample into a platinum crucible and add 5 g of fused borax. Fuse the contents of the crucible over a Teclu or blast burner until complete solution of the mineral is obtained. This is best ascertained by inspection. With many samples a completely transparent melt is obtained, but with certain minerals, such as samarskite, a slight cloudiness of the melt remains after the disappearance of all particles of the oxide sample. The time required for complete decomposition will vary with the degree of grinding of the sample, but not more than 1 hour should be required for titanium minerals, and not more than 2 hours for earth-acid minerals.

When quite cold, transfer the crucible to a polythene beaker, or preferably a deep platinum dish, and add sufficient diluted hydrofluoric acid (1 + 4) to cover the melt. Warm until all soluble salts have passed into solution. Collect the insoluble fluorides on a close-textured filter-paper, such as Whatman No. 40, and wash them well with dilute hydrofluoric acid (1 + 99). Preserve this precipitate for the determination of thorium, rare earths and so on.

To the filtrate add 6 ml of diluted sulphuric acid (1 + 1) and 10 ml of concentrated hydrofluoric acid. Evaporate the solution to copious fumes of sulphuric acid. An infra-red heating lamp, operating as a surface evaporator in a fume cupboard with a good draught, has been found to be the most efficient apparatus for this purpose. The sulphate melt so obtained contains all the titanium, niobium and tantalum originally present in the mineral.

I am indebted to the Director, Geological Survey of Uganda, for permission to publish this Note.

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GEOLOGICAL SURVEY OF UGANDA
P.O. BOX 9
ENTEBBE, UGANDA

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July 11th, 1956

Apparatus

A CONTINUOUS ETHER EXTRACTOR*

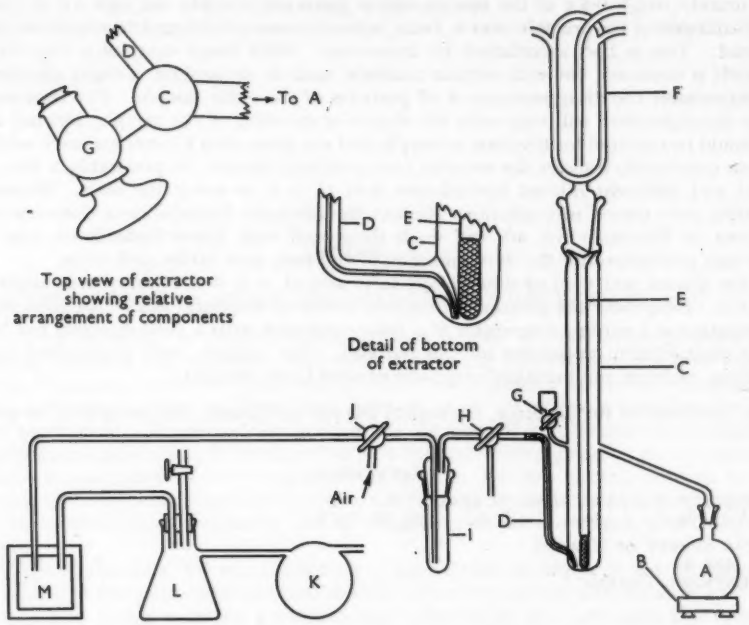
THE solubility of uranium nitrate in organic solvents has been much exploited for the separation and purification of uranium. Many continuous extractors have been described[1,2,3,4] that effectively remove uranium from nitric acid solutions of high and low-grade uranium-bearing materials. In general these extractors suffer from the disadvantage of requiring dismantling after each extraction accompanied by excessive handling by the analyst. This poses serious problems when highly radioactive solutions are involved, especially when it is desirable to recover the raffinate (solution from which the uranium has been extracted). In order to benefit from the inherent advantages of continuous extraction and to minimise the manipulations requiring direct contact by the operator a conventional continuous extractor has been modified to adapt it for use with radioactive solutions.

The continuous extractor is shown schematically in Fig. 1 and in actual laboratory use in Fig. 2. The main features of the modified design include the drain tube, D, and the raffinate aliquot tube, I. Some important advantages are evident not only in the handling of radioactive solutions but also in simplifying the technique of extraction with "cold" solutions. The drain tube, D, permits the radioactive raffinate to be disposed of directly and safely into a shielded active-waste container without dismantling of the apparatus. The drainage is rapid and convenient and

* Based on work performed under the auspices of the U.S. Atomic Energy Commission.

eliminates any possibility of spillage. The raffinate-aliquot tube, I, allows for a positive confirmation that the uranium has been quantitatively extracted. In the apparatus described the inner tube was adjusted so that approximately a 1/20 aliquot of the raffinate remained in the tube, I, after the raffinate had been disposed of. This aliquot was checked spectrographically to ensure that quantitative uranium extraction had been effected. By substituting an appropriately sized flask with standard taper joint for the raffinate aliquot tube, I, the raffinate can be quantitatively recovered for analysis of constituents not extracted.

Although the apparatus can be built to any convenient size, there are several aspects of the design that are critical. The point at which the sample solution enters into the extractor, C, should be just slightly above the point of ether overflow. Experience has proved that, if it is



A = 500-ml flat-bottomed Florence flask with standard 24/40 female joint
B = 4-inch variable temperature-controlled hot-plate
C = extractor, length about 40 cm, made from Pyrex-glass tubing having an outside diameter of 21 mm. The ether overflow arm is attached to the extractor 11 cm from the bottom and is made from 12-mm Pyrex-glass tubing and is about 15 cm long
D = drain tube, made from capillary tubing having an outside diameter of 7 mm and an inside diameter of 1 mm
E = ether-dispersing tube, made from 7-mm Pyrex-glass tubing attached to a coarse sintered-glass filter. The receiving end is fluted to rest on the indented part of the extractor at three points so as to allow the passage of ether vapour
F = Friedrichs condenser with standard 24/40 male joint; 35 cm long
G = sample entrance tube having a volume of about 10 ml, with one hole, a 2-mm stopcock and attached to the extractor with the minimum length of 8-mm tubing
H = three-way stopcock; 2-mm T bore
I = raffinate-aliquot tube; the inner tube is adjusted to a length such that when raffinate is withdrawn from the extractor about 1 ml of the raffinate remains in tube I, thus taking a 1/20 aliquot of the raffinate
J = parallel oblique 2-mm bore stopcock
K = mechanical pump; vented in hood
L = safety bottle, made from a 2-litre suction flask
M = 9-litre shielded liquid-active-waste container

Fig. 1. Schematic diagram of extraction apparatus

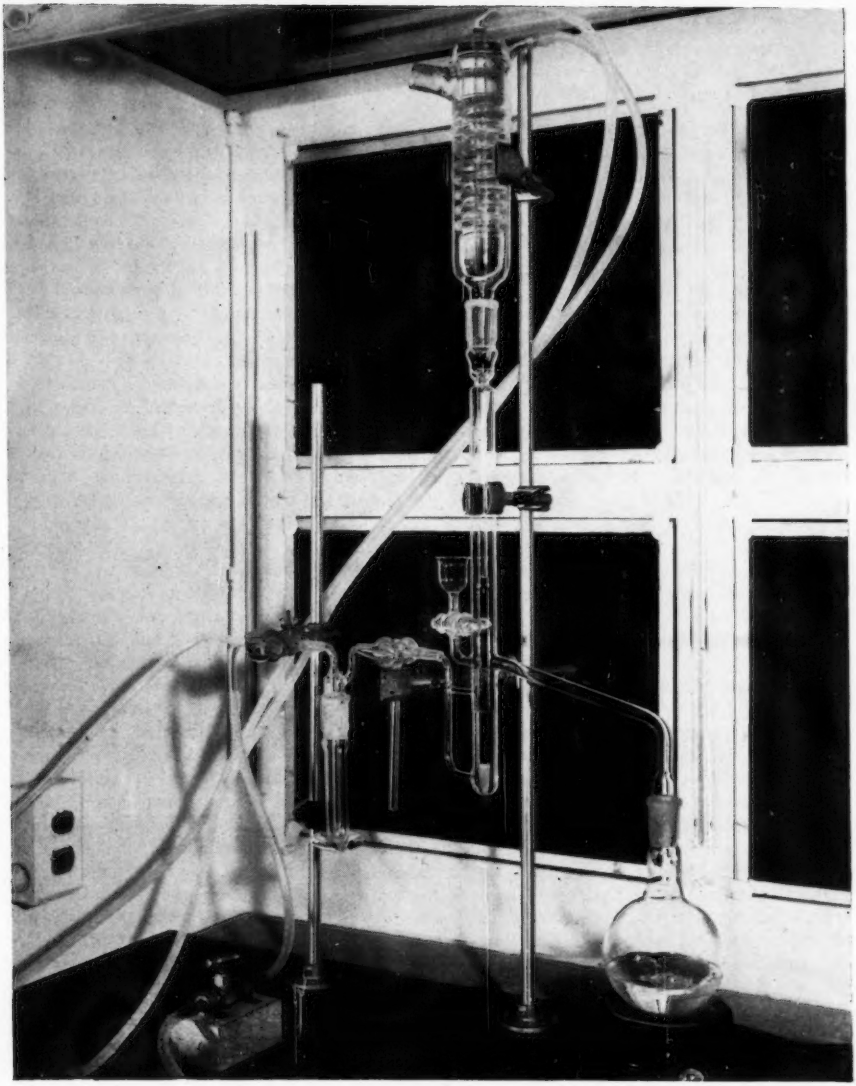


Fig. 2. View of extraction apparatus

[To face p. 68

appreciably higher, the sample solution may flow around the inside of the extractor during introduction and go directly into flask A. The top of the drain tube, D, should be higher than the point of ether overflow, so that if the air supply were inadvertently shut off during extraction, the sample solution would not overflow into the raffinate-aliquot tube, I, and be lost. The ether-dispersing tube must be of such a length as to accommodate a head of ether sufficiently high to force the ether through the coarse sintered-glass dispersing bulb.

OPERATION—

Introduce the uranium sample, in the form of a dried nitrate salt, dissolved in a minimum amount of nitric acid saturated with ammonium nitrate into the extractor via tube G. Wash tube G with saturated ammonium nitrate solution until the volume in the extractor, C, is about 15 ml. Although other nitrate salts such as calcium or aluminium are more effective salting agents,[5] ammonium nitrate has the advantage that if any of the salt is extracted or physically carried over into flask A it will not interfere with the gravimetric determination of the uranium. The decrease in extraction efficiency is of less concern in continuous extraction than in batch extraction. Quantitative recovery is achieved by merely allowing the extraction process to proceed a longer time. Maintain a very small positive air pressure in the apparatus to prevent the sample solution from entering the drain tube, D, and to provide additional mixing during the extraction. Normally the air flow is kept to a minimum, since the globules of ether rising through the solution provide adequate mixing. Place the receiving flask, A, containing 75 ml of water and 75 ml of ether on a small hot-plate, B, adjusted so that the rate of distillation during the extraction is about 8 ml per minute. Extract the sample for 1 hour. Add 1 to 2 ml of concentrated nitric acid saturated with ammonium nitrate at 15-minute intervals to prevent hydrolysis. At the end of the extraction add saturated ammonium nitrate to bring the ether - ammonium nitrate interface almost to the point of the ether overflow, thus making the volume of the aqueous phase in the extractor, C, about 20 ml. Detach the receiving flask, A, from the apparatus, and set it aside for the uranium determination.

Remove the raffinate from the apparatus by connecting the pump, K, to the extractor, C, by means of stopcock J, so that the raffinate is drawn into the shielded liquid-active-waste container, M. Maintain suction until the ammonium nitrate - ether interface just reaches the bottom of the extractor, C. Turn stopcock H at the appropriate moment to prevent an appreciable amount of ether from being drawn into container M. Appreciable amounts of ether drawn into the shielded liquid-active-waste container, M, may cause undesirable side-reactions within the container. Also, if any ether is collected in tube I, a representative sample of the raffinate would not be obtained. After turning stopcocks H and J so that air is again flowing through the extractor, C, detach tube I containing about 1 ml of the raffinate and reserve the contents for a check on the completeness of uranium extraction. Replace a clean tube, I, and the apparatus is ready for another extraction.

The extracted uranium can be determined gravimetrically by precipitation with ammonium hydroxide and ignition to U_3O_8 at 850° C. Although a large degree of decontamination is achieved, the extracted uranium still contains appreciable amounts of activity. Therefore, it is necessary to observe the usual precautions for handling radio-active materials when determining the uranium gravimetrically. The completeness of the extraction can be verified by determining uranium in the aliquot of the raffinate remaining in tube I spectrographically,[6] colorimetrically[7] or fluorophotometrically.[8] Even though it was shown that the extraction conditions described above will quantitatively extract 200 to 300-mg amounts of uranium, checking for completeness of extraction by some method is the only positive way to assure oneself that no unknown interference in the sample, or inadvertent use of non-saturated ammonium nitrate, has prevented the quantitative extraction of the uranium. In practice it was ascertained spectrographically that the amount of uranium remaining in the approximately 1/20 aliquot of the original sample was less than 10 μg. This means that the extraction is better than 99·9 per cent. complete when the uranium extracted is 200 mg or more.

This apparatus and procedure has been used to determine uranium in two artificial-standard samples containing about 18·70 and 200·0 mg of uranium per ml, respectively, and in a number of solutions of irradiated uranium metal with concentrations ranging from 58 to 335 mg of uranium per ml. The irradiated samples contained levels of β and γ fission-product activity ranging from a few hundred milliroentgens to several roentgens per hour. The results shown in Table I indicate the efficiency of the apparatus for the separation of uranium and the degree of accuracy and precision of the gravimetric determination itself. The precision attained on 21 solutions of Pile-irradiated aluminium-jacketed uranium slugs analysed at least in duplicate was found to be statistically the same.

TABLE I

RESULTS FOR STANDARD URANIUM SOLUTIONS

Solution I,* mg per ml	Solution II,† mg per ml			
18·70	200·2	199·8	199·5	199·8
18·72	199·9	199·9	199·9	199·9
18·73	199·9	200·2	199·8	199·5
18·70	199·9	199·7	199·4	
Average = 18·72(3)	Average = 199·8(2)			

Standard deviation = 0·22 mg

* Prepared at Argonne National Laboratory from MS–ST primary standard[9] U_3O_8 to contain 18·70 mg of uranium per ml.

† Standard solution prepared at a co-operating Atomic Energy Commission laboratory to contain 200·0 mg of uranium per ml.

Although this apparatus has been used extensively to separate uranium from large amounts of fission-product activity, it can also be used conveniently to separate uranium from various non-radioactive solutions. For example it has been successfully applied to the separation of uranium from uranium - silver - calcium - magnesium mixtures and uranium - rare-earth mixtures that have a low cerium content. The design is particularly advantageous for the recovery of a constituent in the raffinate, since the raffinate can be quantitatively obtained without dismantling the apparatus. A case in point is the analysis of uranium and aluminium mixtures for the two elements. The extractor permits the simple and easy isolation of uranium and aluminium for their subsequent determination.

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Ministry of Agriculture, Fisheries and Food

STATUTORY INSTRUMENT*

1956—No. 1853. The Fertilisers and Feeding Stuffs (Amendment) Regulations, 1956. Price 5d.

These Regulations, which came into operation on January 1st, 1957, make provision for the use of the Wilson quinoline - molybdate method of analysis for the determination of phosphoric acid (P_2O_5) *as an official alternative method to that in the principal Regulations* (*S.I.*, 1955, *No.* 1673; *Analyst*, 1955, **80**, 909), *and also correct two typographical errors therein.*

FOOD STANDARDS COMMITTEE

SECOND REPORT ON PROCESSED CHEESE AND CHEESE SPREAD

THE Minister of Agriculture, Fisheries and Food, the Minister of Health and the Secretary of State for Scotland have approved for publication a Report of the Food Standards Committee on Processed Cheese and Cheese Spread making recommendations about composition.

This Report takes account of representations made after the publication of the Committee's first report in 1949. Any observations from interested parties should be submitted to the Assistant Secretary, Food Standards and Hygiene Division, Ministry of Agriculture, Fisheries and Food, Great Westminster House, Horseferry Road, London, S.W.1, to arrive not later than January 31st, 1957.

Copies of the Report may be obtained from H.M. Stationery Office, price 6d. (plus postage).

* Obtainable from H.M. Stationery Office. Italics indicate changed wording.

British Standards Institution

AMENDMENT SLIPS*

PRINTED slips bearing amendments to British Standards have been issued by the Institution, as follows—

PD 2627—Amendment No. 1 (November, 1956) to B.S. 894:1956. Ubbelohde Apparatus for Flow and Drop Points.

PD 2640—Amendment No. 4 (November, 1956) to B.S. 1425:1954. Cleanliness of Fillings and Stuffings.

* Obtainable from the British Standards Institution, Sales Department, 2 Park Street, London, W.1.

Publications Received

SPOT TESTS IN ORGANIC ANALYSIS. By Fritz Feigl, Eng., D.Sc. Translated by RALPH E. OESPER, Ph.D. Fifth English edition. Pp. xx + 616. Amsterdam, London and New York: Elsevier Publishing Company; London: Cleaver-Hume Press Ltd. 1956. Price $10.00; 55s.

THE EXAMINATION OF NEW ORGANIC COMPOUNDS. By WALTER T. SMITH, jun., and RALPH L. SHRINER. Pp. viii + 136. New York: John Wiley & Sons Inc.; London: Chapman & Hall Ltd. 1956. Price $3.50; 28s.

THE REACTIVE INTERMEDIATES OF ORGANIC CHEMISTRY. By JOHN E. LEFFLER. Pp. x + 275. New York and London: Interscience Publishers Inc. 1956. Price $6.00; 45s.

CALENDAR OF THE PHARMACEUTICAL SOCIETY OF GREAT BRITAIN, 1956–1957. Pp. vi + 309. London: The Pharmaceutical Press. 1956. Price 17s. 6d.

A GUIDE TO QUALITATIVE ORGANIC CHEMICAL ANALYSIS. By R. P. LINSTEAD, C.B.E., D.Sc., D.I.C., F.R.I.C., F.R.S., and B. C. L. WEEDON, D.Sc., Ph.D., A.R.C.S., D.I.C., F.R.I.C. Pp. xii + 169. London: Butterworths Scientific Publications; New York: Academic Press Inc. 1956. Price 21s.; $4.50.

OXINE AND ITS DERIVATIVES. Volume III: DERIVATIVES OF OXINE—PART 1. By R. G. W. HOLLINGSHEAD, M.A., A.R.I.C. Pp. vi + 617–896 + Index to vol. III, pp. 1–16. London: Butterworths Scientific Publications. 1956. Price 42s.

OXINE AND ITS DERIVATIVES. Volume IV: DERIVATIVES OF OXINE—PART 2. By R. G. W. HOLLINGSHEAD, M.A., A.R.I.C. Pp. vi + 897–1212 + Index to vols. I–IV, pp. 1–40. London: Butterworths Scientific Publications. 1956. Price 42s.

STERIC EFFECTS IN ORGANIC CHEMISTRY. Edited by MELVIN S. NEWMAN, Ph.D. Pp. viii + 710. New York: John Wiley & Sons Inc.; London: Chapman & Hall Ltd. 1956. Price $12.50; 100s.

METHODS OF CHEMICAL ANALYSIS AS APPLIED TO SEWAGE AND SEWAGE EFFLUENTS. Issued by the Ministry of Housing and Local Government. Pp. vi + 96. London: Her Majesty's Stationery Office. 1956. Price 10s. 6d.

REPORTS ON THE PROGRESS OF APPLIED CHEMISTRY. Volume XL: 1955. Editor: H. S. ROOKE, M.Sc., F.R.I.C. Pp. 1000. London: The Society of Chemical Industry. 1956. Price 60s.

AMINO ACID HANDBOOK: METHODS AND RESULTS OF PROTEIN ANALYSIS. By RICHARD J. BLOCK, Ph.D., and KATHRYN W. WEISS, A.B., and others. Pp. xiv + 386. Illinois, U.S.A.: Charles C. Thomas, Publisher; Oxford: Blackwell Scientific Publications. 1956. Price 80s.

Errata

FEBRUARY (1956) ISSUE, p. 108, 44th line. *For* "40 g" *read* "40 μg."

DECEMBER (1956) ISSUE, p. 680, 14th and 15th lines of Summary of "General Review of Sub-micro Methods." *For* "were not generally applicable to the ultra-micro scale of working" *read* "were not generally applicable to the series of ultra-micro analyses being investigated."

THE SOCIETY FOR ANALYTICAL CHEMISTRY

FORMERLY THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

FOUNDED 1874. INCORPORATED 1907.

THE objects of the Society are to encourage, assist and extend the knowledge and study of analytical chemistry by holding periodical meetings, by promoting lectures, discussions and conferences, and by the publication of a journal devoted to all branches of analytical chemistry; to study questions relating to the analysis, nature and composition of natural and manufactured materials generally; and to promote, or assist to promote, the efficiency and the proper administration of the laws relating to the control and composition of such materials.

The Society includes members of the following classes:—(*a*) Ordinary Members who are persons of not less than 21 years of age and who are or have been engaged in analytical, consulting or professional chemistry; (*b*) Junior Members who are persons between the ages of 18 and 27 years and who are or have been engaged in analytical, consulting or professional chemistry or *bona fide* full-time or part-time students of chemistry. Each candidate for election must be proposed by three Ordinary Members of the Society who shall provide written testimony of their personal knowledge as to his scientific and professional fitness. If the Council in their discretion think fit, such testimony may be dispensed with in the case of a candidate not residing in the United Kingdom. Every application is placed before the Council and the Council have the power in their absolute discretion to elect candidates or to suspend or reject any application. Subject to the approval of Council, any Junior Member above the age of 21 may become an Ordinary Member if he so wishes. A member ceases to be a Junior Member on the 31st day of December in the year in which he attains the age of 27 years. Junior Members may attend all meetings, but are not entitled to vote.

The Entrance Fee for Ordinary Members is £1 1s. and the Annual Subscription is £3 3s. Junior Members are not required to pay an Entrance Fee and their Annual Subscription is £1 1s. No Entrance Fee is payable by a Junior Member on transferring to Ordinary Membership. The Entrance Fee (where applicable) and first year's Subscription must accompany the completed Form of Application for Membership. Subscriptions are due on January 1st of each year.

Scientific Meetings of the Society are usually held on the first Wednesday in October, November, December, February, April and May, in London, but from time to time meetings are arranged in other parts of the country. The Annual General Meeting is usually held in London on the first Friday in March. Notices of all meetings are sent to members by post.

All members of the Society have the privilege of using the Library of The Chemical Society. Full details about this facility can be obtained from the Librarian, The Chemical Society, Burlington House, Piccadilly, London, W.1.

The Analyst, the official organ of the Society, is issued monthly, to all Ordinary and Junior Members, and contains reports of the proceedings of the Society, original papers and notes, information about analytical methods, Government reports and reviews of books. In addition, all Ordinary Members receive *Analytical Abstracts*, providing a reliable index to the analytical literature of the world.

Forms of application for membership of the Society may be obtained from the Secretary, The Society for Analytical Chemistry, 14 Belgrave Square, London, S.W.1.

LOCAL SECTIONS AND SUBJECT GROUPS

THE North of England, Scottish, Western and Midlands Sections were formed to promote the aims and interests of the Society among the members in those areas. The Microchemistry, Physical Methods and Biological Methods Groups have been formed within the Society to further the study of the application of microchemical, physical and biological methods of analysis. All members of the Society are eligible for membership of the Groups.

The Sections and Groups hold their own meetings from time to time in different places. There is no extra subscription for membership of a Section or Group. Application for registration as a member should be made to the Secretary.